

## ARTICLE

# Influence of 4-week or 12-week glucocorticoid treatment on metabolic changes in patients with active moderate-to-severe thyroid-associated ophthalmopathy

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## Abstract

Thyroid-associated ophthalmopathy (TAO) is a serious, progressive, vision-threatening and difficult-to-treat organ-specific autoimmune disease. The course, therapeutic effects and prognosis of moderate to severe TAO vary greatly. High-dose intravenous glucocorticoid (IVGC) therapy is considered a first-line treatment for active moderate-to-severe TAO, but there is still insufficient evidence regarding the treatment duration. Long-term IVGC therapy can influence the metabolism of glucose, lipids, and bone. This study was designed to compare changes in metabolic and immunological indexes as well as the magnetic resonance imaging apparent diffusion coefficient (ADC) of the extraocular muscles after 4 and 12 weeks of IVGC

**Abbreviations:** ADC, apparent diffusion coefficient; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; CAS, clinical activity score; CD, cluster of differentiation; Cr, creatinine; CTX, carboxyterminal telopeptide of type I collagen; DWI, diffusion-weighted imaging; EOM, extraocular muscles; FOV, field of view; FPG, fasting plasma glucose; GD, Graves' disease; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IR, inferior rectus; IVGC, intravenous glucocorticoid; LDL, low-density lipoprotein; LR, lateral rectus; MR, medial rectus; NA, number of sample acquisitions; OC, osteocalcin; PINP, aminoterminal propeptide of type I collagen; SMG, superior muscle group; TAO, thyroid-associated ophthalmopathy; TC, total cholesterol; TF, turbo factor; TG, triglycerides; TGAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSE, turbo spin-echo; UA, uric acid; VS, voxel size.

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therapy. Forty-eight patients with active moderate-to-severe TAO were included in this retrospective cohort study. Metabolism and immunological indexes were measured before and after therapy. The ADC and clinical activity score (CAS) were used to evaluate the efficacy of treatment in these patients. We found that the patients in the 12-week group had increased fasting plasma glucose ( $p = 0.004$ ), glycated hemoglobin ( $p = 0.028$ ), total cholesterol ( $p < 0.001$ ), and low-density lipoprotein ( $p < 0.001$ ) after therapy. The patients in both groups had reduced bone metabolism markers after therapy. Thyroid peroxidase antibody and thyrotropin receptor antibody levels decreased after treatment in both groups ( $p < 0.001$ ). A significant decrease in thyroglobulin antibody levels was found in the 4-week group ( $p = 0.006$ ). The change in the ADC was higher in the 4-week group than in the 12-week group ( $p = 0.014$ ). However, there were no significant differences in CAS values between the two groups. Therefore, 4-week IVGC therapy was recommended for patients with TAO with glucose and lipid disorders.

### Study Highlights

#### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

High-dose intravenous glucocorticoid treatment has been considered a first-line treatment for active moderate-to-severe thyroid-associated ophthalmopathy (TAO). At present, the 12-week therapy protocol with a total dose of 4.5 g of methylprednisolone is widely used. This program has a long duration of medication and causes many metabolic side effects.

#### WHAT QUESTION DID THIS STUDY ADDRESS?

The 4-week or 12-week 4.5 g glucocorticoid (GC) therapy protocol, which one has better efficacy and less side effects for active moderate-to-severe TAO?

#### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The 4-week protocol could significantly improve the apparent diffusion coefficient (ADC) in extraocular muscles on magnetic resonance imaging (MRI) by 1.5 times that of the 12-week protocol and it seems to have less impact on metabolism of glucose and lipids.

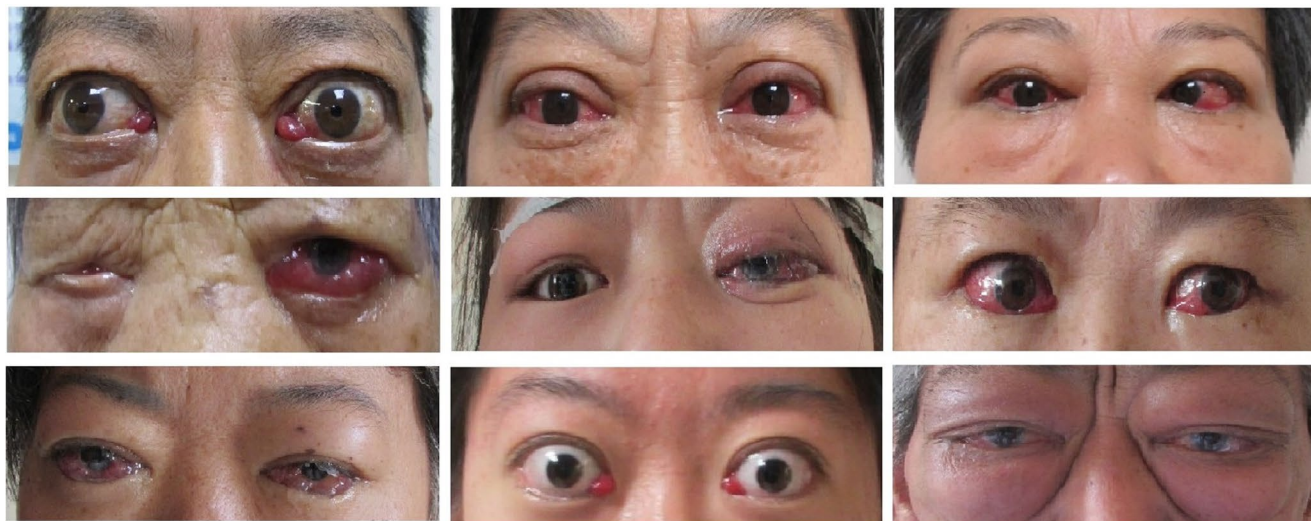
#### HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

The ADC of MRI as an objective evaluation index of the severity of extraocular muscles inflammation in patients with TAO can be popularized clinically. Moreover, the 4-week GC therapy protocol is recommended for patients with glucose and lipid disorders to standardize GC therapy programs for active moderate-to-severe TAO.

## INTRODUCTION

Graves' disease (GD) is a complex autoimmune thyroid disease that is caused by multiple factors, such as genetic, environmental, and immune disorders.<sup>1</sup> A meta-analysis of European studies showed that the incidence rate of GD is 51 cases per 100,000 per year.<sup>2</sup> It is estimated that 30–50% of patients with GD experience thyroid-associated ophthalmopathy (TAO),<sup>1</sup> which is an autoimmune inflammatory disease that affects the orbit.<sup>3</sup> The clinical manifestations of TAO include

eye pain, eyelid swelling, photophobia, tearing, diplopia, exophthalmos, eyelid contracture, limitation of eye movement, optic nerve dysfunction, foreign body sensation, blurred vision, visual deterioration, and even blindness, which are caused by inflammation-induced fibrosis of the ocular muscles and adipogenesis<sup>4–6</sup> (Figure 1). These symptoms cause great distress to patients, which affects their quality of life (QOL) and mental health. Although many treatment methods have been proposed, the clinical efficacy has been unsatisfactory.<sup>7</sup> Frequently, QOL is greatly impaired in patients with TAO.<sup>8</sup>



**FIGURE 1** The patients with active moderate and severe thyroid-associated ophthalmopathy (TAO) before treatment with symptoms of 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 plica, and conjunctival edema

The clinical activity score (CAS) has been widely used to grade disease activity, with a score of three or more reflecting the activity of TAO. However, the CAS is a subjective scoring system. Other quantitative methods, such as magnetic resonance imaging (MRI), are still needed to evaluate TAO. Apparent diffusion coefficients (ADCs) were calculated by diffusion-weighted imaging (DWI) in MRI. Researchers have found that the ADC values of the extraocular muscles (EOMs) are higher in patients with TAO than in healthy controls. Therefore, the ADC might be an objective measure of the activity and severity of EOMs in patients with TAO.<sup>9</sup>

High-dose intravenous glucocorticoid (IVGC) treatment has been considered a first-line treatment for active moderate-to-severe TAO. In a previous study, 12-week therapy (6 weekly infusions of 0.5 g, followed by 6 weekly infusions of 0.25 g) with IVGC was more efficient and safer than 4-week therapy (daily infusion of 0.5 g for 3 consecutive days for 2 weeks, followed by a daily infusion of 0.25 g for 3 consecutive days for 2 weeks) for patients with active moderate-to-severe TAO.<sup>10</sup> Subsequently, the 12-week treatment regimen was recommended by the European Group on Graves' Orbitopathy (EUGOGO) for most cases of active moderate-to-severe TAO.<sup>11</sup> However, there have been no relevant studies on metabolic alterations after 4-week and 12-week therapy.

IVGC could influence the metabolism of glucose, lipids, and bone. In this study, we compared metabolic and immunological indexes between groups receiving 4-week and 12-week therapy. Furthermore, we also compared the changes in CAS and ADC of the EOMs after therapy to evaluate the efficacy in these two groups.

## METHODS

### Study design and participants

This was a retrospective cohort study of patients with TAO admitted to the Shanghai Ninth People's Hospital affiliated to Shanghai Jiaotong University School of Medicine from January 2016 to December 2018.

The inclusion criteria are as follows. The more severe side of the eye was diagnosed as active moderate-to-severe TAO. The diagnosis was based on the Bartley diagnostic criteria.<sup>12</sup> Activity and severity assessments of TAO were based on the EUGOGO consensus statement.<sup>3</sup> Patients with a CAS greater than or equal to 3/7 were considered to have active TAO. Patients with moderate-to-severe TAO usually have any one or more of the following: lid retraction greater than or equal to 2 mm, moderate or severe soft tissue involvement, exophthalmos greater than or equal to 3 mm, and inconstant or constant diplopia.<sup>3</sup>

The exclusion criteria were as follows: severe heart, liver, and renal insufficiency, such as myocardial ischemia or myocardial infarction, arrhythmia and cardiac insufficiency; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) greater than or equal to 1.5 times the upper limit of the normal value; and estimated glomerular filtration rate less than  $60 \text{ ml/min} \cdot (1.73 \text{ m}^2)^{-1}$ . In addition, patients with gastric ulcers, gastric bleeding, diabetes, other autoimmune diseases, uncontrolled hypertension, tuberculosis, mental illness, and glaucoma; patients with a positive pregnancy test or who were breastfeeding; patients who received radioactive iodine treatment or a hepatitis vaccine within 3 months before enrollment; patients with ophthalmological signs caused

by other diseases; patients who had received systemic immunotherapy for Graves' orbitopathy (GO) within 1 month before enrollment, including oral or IVGC, or other immunosuppressants; and patients who had orbital radiotherapy were excluded.

The study protocol was approved by the ethics committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine (Ethical approval number: SH9H-2019-T301-1). The study protocol conformed with the ethical guidelines of the 1975 Declaration of Helsinki, as reflected by the a priori approval by the appropriate institutional review committee. Informed consent was obtained from all participants included in the study.

Data were collected from a total of 205 patients with TAO with 4.5 g IVGC therapy. Forty-eight patients met the final selection criteria. Twenty-six and 22 patients received 4.5 g IVGC for 12 weeks (6 weekly infusions of 0.5 g, followed by 6 weekly infusions of 0.25 g) and 4 weeks (daily infusions of 0.5 g for 3 consecutive days per week for 2 weeks, followed by a daily infusion of 0.25 g for 3 consecutive days per week for 2 weeks), respectively.<sup>13</sup>

## Measurements

Ophthalmic assessments, ocular enhanced MRI examinations, blood sample collection, and questionnaire completion were performed on the day before the first week of treatment and on the day after the last treatment.

A questionnaire about sociodemographic characteristics, medical history, family history, and lifestyle factors was administered during the interview. A single endocrinologist conducted the interviews and clinical examinations, including weight, height, and blood pressure measurements, according to a standard protocol. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared. Current smoking was defined as having smoked at least 100 cigarettes in one's lifetime and currently smoking cigarettes.<sup>14</sup>

Blood samples were obtained between 6:00 a.m. and 9:00 a.m. after fasting for at least 8 hours. Blood was immediately refrigerated after phlebotomy, and after 2 hours, it was centrifuged, and the serum was aliquoted and frozen in a central laboratory. Fasting plasma glucose (FPG), serum creatinine, serum urea nitrogen, serum uric acid, triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alkaline phosphatase (ALP), ALT, and AST levels were determined with an ADVIA2400 (Siemens). Glycated hemoglobin (HbA1c) was measured with a TOSOH HLC-723 G8. Serum total T3 (TT3), total T4 (TT4), free T3 (FT3), free T4 (FT4), thyroid-stimulating hormone (TSH), and thyroid peroxidase antibodies (TPOAbs) were measured with a BECKMAN COULTER

UniCel DxI800. Thyrotropin receptor antibody (TRAb), thyroglobulin antibody (TGA), serum osteocalcin (OC), amino terminal propeptide of type I collagen (PINP), and carboxy-terminal telopeptide of type I collagen (CTX) were measured with a Cobas 6000 (Roche). The detection of the proportion of the cluster of differentiation (CD) series in peripheral blood was conducted with a BD FACSCanto II flow cytometry system. The detection of immunoglobins, such as IgG, was conducted by immunoturbidimetry.

Ophthalmic assessments were jointly performed by an endocrinologist and an ophthalmologist. The CAS (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, and conjunctival edema) was evaluated as previously reported.<sup>15</sup>

## MRI assessment of GO

We performed orbital MRI in all patients after evaluating the CAS. There was an average of 1–2 days between the CAS evaluation and MRI. During the MRI examination, patients closed their eyes in the primary position and minimized eye movements to prevent the movement and contraction of the EOM. We obtained coronal DWI images before contrast administration using the same T1-weighted (T1W) sequence. MRI was performed on a 3.0-T MRI system (Philips Ingenia) with a head coil. The routine orbital MRI protocol included an axial T1W turbo spin-echo (TSE) sequence with 3-mm slice thickness, repetition time (TR; ms)/echo time (TE; ms) = 497.7/8.0, turbo factor (TF) = 3, voxel size (VS; mm) = 0.55 × 0.69 × 3, number of sample acquisitions (NAs) = 2, matrix = 220 × 210 × 15 pixels and field of view (FOV) = 120 × 150 × 49 mm; axial T2-weighted (T2W) TSE spectral presaturation with inversion recovery sequences with slice thickness = 3 mm, TR (ms)/TE (ms) = 3000.0/80.0, TF = 17, VS = 0.5 × 0.65 × 3, NA = 1.6, matrix = 240 × 226 × 15 pixels and FOV = 120 × 150 × 49 mm; coronal T2W driven equilibrium (DRIVE) sequence with slice thickness = 3.5 mm, TR (ms)/TE (ms) = 3000.0/90.0, TF = 15, VS = 0.5 × 0.61 × 3.5, NA = 1.6, matrix = 240 × 242 × 20 pixels, and FOV = 120 × 160 × 77 mm; coronal DWI sequence with TR (ms)/TE (ms) = 4055.8/71.4, FOV = 220 × 183 × 63 mm, matrix size = 124 × 91 × 16 pixels, NA = 4, slice thickness = 3 mm, and b-values of 0 and 1000 s/mm<sup>2</sup>. The ADC maps were automatically reconstructed by commercially available software and calculated in \*10<sup>-3</sup> mm<sup>2</sup> s<sup>-1</sup>.

Because the superior rectus muscle, the superior ophthalmic vein, and the levator palpebrae superioris are difficult to distinguish from each other, they were measured together as the superior muscle group (SMG).

On the basis of a study conducted by Bijlsma and Mourits, the measurements of ADC values of the SMG, medial rectus,

lateral rectus, and inferior rectus were made from the thickest part of the muscle in selected coronal planes from both side muscles<sup>16</sup> (Figure 2). The ADC values of each muscle were measured by a radiologist who was unaware of the clinical history of the participants. The  $\Delta$ ADC-max value was the maximum value of the pretreatment ADC minus the post-treatment ADC in eight muscles.

## Statistical analysis

Data analyses were performed using IBM SPSS Statistics, version 23 (IBM Corporation, Armonk, NY). A  $p$  value less than 0.05 indicated significance (2 sided). Continuous variables are summarized as the mean  $\pm$  SD or median (interquartile range), and categorical variables are summarized as percentages (%). A  $t$ -test was used to compare two groups or compare the values before and after treatment for continuous variables. If a continuous variable was not normally distributed, log10 normal transformation was performed before conducting the statistical analysis. If the log10 normal transformation was still not normally distributed, a Mann-Whitney  $U$  test or F-Wilcoxon test was performed. The  $\chi^2$  test was used to compare proportions. The parameters labeled with  $\Delta$  indicate the difference between a value before treatment minus a value after treatment.

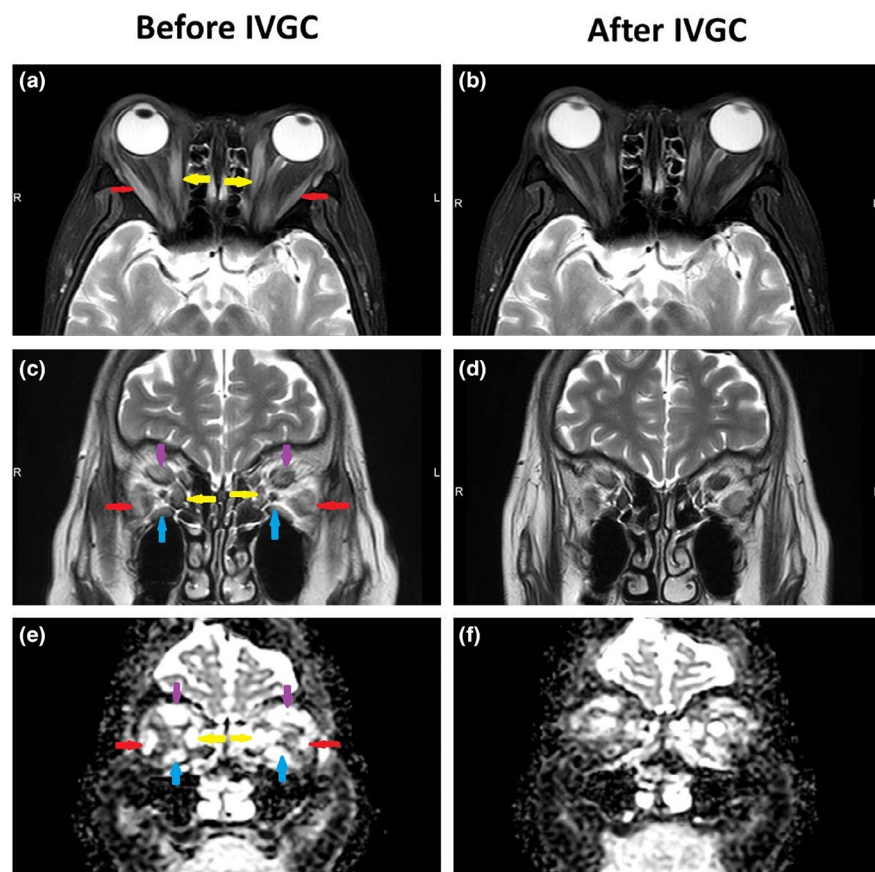
## RESULTS

### Characteristics of the participants

The general demographic characteristics of the study population are shown in Table 1. The 12-week group included 26 patients, with a mean age of 57 years old and a median duration of eye symptoms of 6.5 months. The 4-week group included 22 patients, with a mean age of 55 years old and a median duration of eye symptoms of 8.0 months. There were no significant differences in age, duration of eye symptoms, smoking proportion, BMI, sex, FPG, HbA1c, thyroid function, bone turnover markers, or immunologic indexes between the two groups before the therapeutic intervention ( $p > 0.05$ ). No patients developed gastric bleeding (including hematemesis, hematochezia, melena, or positive fecal occult blood tests) after IVGC therapy.

### Comparison of metabolic indicators before and after treatment in both groups

Table 1 summarizes the comparison of metabolic indicators before and after treatment for both protocols. The patients had higher FPG ( $p = 0.004$ ), HbA1c ( $p = 0.028$ ), total cholesterol ( $p < 0.001$ ), and LDL-C ( $p < 0.001$ ) after 12-week



**FIGURE 2** The changes of magnetic resonance imaging after intravenous glucocorticoid (IVGC) treatment in patients with active moderate and severe thyroid-associated ophthalmopathy. Transversal fat-saturated T2 weighted (a, b), coronal fat-saturated T2 weighted (c, d) and apparent diffusion coefficient (e, f) maps in the same plane of orbits in the patient. The measurement of apparent diffusion coefficient values in the superior muscle group (SMG), medial rectus (MR), lateral rectus (LR) and inferior rectus (IR) was made from the thickest part of the muscle in selected coronal planes from both side muscles. The measured area is approximately 10 mm. The red arrows represent LR, the yellow arrows represent MR, the purple arrows represent SMG, the blue arrows represent IR

**TABLE 1** Characteristics of the participants

	12 week		4 week	
	Baseline	After treatment	Baseline	After treatment
No.	26		22	
Age, <sup>a</sup> year	57.2 ± 12.5		55.1 ± 12.1	
Male, <sup>b</sup>	14 (53.8%)		13 (59.1%)	
Smoking, <sup>b</sup>	9 (34.6%)		7 (31.8%)	
Duration of eye symptoms, <sup>c</sup> month	6.5 (3.0, 14.0)		8.0 (5.0, 12.0)	
Antithyroid medication, <sup>b</sup> %	54.5		65.0	
BMI, <sup>a</sup> kg/m <sup>2</sup>	23.58 ± 3.65	23.69 ± 3.37	23.71 ± 2.95	22.62 ± 2.97
ALT, U/L	15.0 (11.5, 29.0)	16 (11.5, 23.75)	17.5 (13.3, 27)	21.5 (14.75, 45)
AST, U/L	17.0 (13.5, 23.0)	16.0 (13.0, 20.5)	18 (15, 19.75)	15 (11.5, 20.25)
BUN, mmol/L	5.33 ± 1.06	5.60 ± 1.26	5.76 ± 2.10	6.57 ± 1.98
Cr, umol/L	82.0 (69.5, 102.5)	90.0 (77.0, 99.5)	84.0 (72.5, 94.0)	83.0 (73.0, 95.0)
UA, umol/L	305 (256.5, 433.5)	285 (256, 349.5)	287 (251.3, 383.5)	219 (179, 296)
Glycolipid metabolism				
FPG, <sup>a</sup> mmol/l	5.05 ± 0.59	6.29 ± 2.15*	5.66 ± 1.44	6.45 ± 1.90
HbA1c, <sup>a</sup> %	5.75 ± 0.52	6.15 ± 0.91**	6.10 ± 0.78	6.88 ± 1.55
TC, <sup>a</sup> mmol/l	4.46 ± 0.88	5.81 ± 1.26***	4.85 ± 1.30	5.40 ± 1.25
TG, <sup>d</sup> mmol/l	1.50 (1.09, 2.25)	1.32 (0.90, 2.07)	1.27 (1.02, 1.55)	1.24 (0.97, 1.52)
HDL, <sup>a</sup> mmol/l	1.25 ± 0.40	1.66 ± 0.44***	1.21 ± 0.35	1.63 ± 0.51***
LDL, <sup>a</sup> mmol/l	2.99 ± 0.74	4.05 ± 1.31***	3.48 ± 1.13	3.65 ± 1.03
Thyroid function				
TSH, <sup>d</sup> μU/ml	0.06 (0.02, 1.99)	2.88 (0.47, 6.49)**	0.92 (0.03, 6.92)	0.47 (0.10, 1.45)
TT4, <sup>d</sup> μg/dL	7.97 (6.8, 9.02)	6.86 (6.04, 7.89)*	7.81 (5.24, 9.54)	7.15 (6.04, 7.97)
TT3, <sup>d</sup> ng/ml	0.98 (0.89, 1.27)	0.87 (0.72, 0.93)*	0.99 (0.88, 1.21)	0.74 (0.60, 0.90)***
FT4, <sup>d</sup> ng/dL	0.89 (0.69, 1.11)	0.75 (0.68, 0.81)**	0.77 (0.59, 1.07)	0.83 (0.66, 0.87)
FT3, <sup>d</sup> pg/ml	3.43 (3.11, 3.83)	3.03 (2.70, 3.39)***	3.38 (3.11, 3.71)	3.00 (2.47, 3.43)**
TPOAb, <sup>d</sup> IU/ml	33.65 (2.1, 223.23)	10.25 (1.28, 43.28)***	1.60 (0.70, 151.58)	1.55 (0.45, 59.13)***
TGAb, <sup>d</sup> IU/ml	12.67 (10, 52.67)	11.96 (10.29, 25.48)	12.69 (10.56, 33.73)	10.41 (10.00, 13.96)*
TRAb, <sup>d</sup> IU/L	13.83 (3.74, 40.00)	2.51 (1.52, 15.47)***	12.52 (5.12, 30.75)	5.16 (2.08, 14.34)***
Bone turnover markers				
OC, <sup>c</sup> ng/ml	26.44 (15.65, 39.16)	25.00 (11.02, 37.89)*	25.53 (18.90, 32.40)	10.84 (6.89, 24.26)***
PINP, <sup>c</sup> ng/ml	64.68 (50.55, 146.40)	51.08 (21.37, 97.74)***	91.37 (70.34, 125.85)	41.84 (25.74, 62.21)*
CTX, <sup>a</sup> ng/ml	1.19 ± 0.64	0.68 ± 0.49***	0.77 ± 0.27	0.65 ± 0.25**
ALP, U/L	89.38 ± 34.50	88.25 ± 34.33	88.56 ± 28.62	81.13 ± 22.72
Immunologic index				
CD3+T cell, %	68.45 ± 7.57	62.35 ± 11.26	66.85 ± 10.38	57.49 ± 13.66
CD3+CD4+ T cell, %	44.38 ± 7.38	39.22 ± 11.53	37.87 ± 11.46	31.22 ± 9.59
CD3+CD8+T cell, %	22.82 ± 7.32	24.11 ± 10.18	26.36 ± 11.74	23.92 ± 8.84
CD4+/CD8+ T cell, %	2.22 ± 1.02	1.91 ± 0.98	1.95 ± 1.88	1.45 ± 0.56
CD19+ B cell, %	17.85 ± 6.01	17.05 ± 6.18	16.42 ± 5.55	22.62 ± 7.69
CD16/CD56+ NK cell, %	11.97 ± 6.84	19.17 ± 11.10	15.50 ± 8.68	18.73 ± 10.35
IgG	11.05 ± 3.67	8.37 ± 8.33	11.23 ± 2.59	8.10 ± 1.78
IgM	1.05 (0.82, 1.56)	1.09 (0.83, 1.51)	1.17 (0.74, 1.64)	1.07 (0.71, 1.61)

(Continues)

**TABLE 1** (Continued)

	12 week		4 week	
	Baseline	After treatment	Baseline	After treatment
IgA	2.30 ± 1.02	1.86 ± 0.85	2.30 ± 0.72	1.73 ± 0.52
IgE	46.70 (24.98, 112.50)	27.90 (17.83, 55.08)	24.30 (17.70, 75.40)	39.00 (17.70, 93.83)
CAS				
CAS (right eye)	3 (3, 5)	1 (0, 1)	4 (2, 5)	1 (0, 1)
CAS (left eye)	3 (2, 4)	1 (0, 1)	4 (2, 5)	1 (0, 1)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; B-CTX, Beta-Crosslaps; BMI, body mass index; BUN, blood urea nitrogen; CAS, clinical activity score; Cr, creatinine; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OC, osteocalcin; PINP, type I procollagen amino terminal extension peptide; TC, total cholesterol; TG, triglycerides; TGA, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; UA, uric acid.

Data are expressed as mean ± SD, number (percentage), or median (interquartile interval).

<sup>a</sup>T-test.

<sup>c</sup>Log10 normal transformed before *t*-test.

<sup>d</sup>F-Wilcoxon test.

\*0.001 < *p* ≤ 0.01 vs. corresponding before treatment group.

\*\*0.01 < *p* < 0.05 vs. corresponding before treatment group.

\*\*\**p* ≤ 0.001 vs. corresponding before treatment group.

<sup>b</sup>Chi-square test.

IVGC treatment. There were no significant differences after the 4-week IVGC treatment. The patients in both groups had lower OC, PINP, and CTX after treatment.

Regarding thyroid function, TSH levels increased and TT4, TT3, FT4, and FT3 levels decreased after therapy in the 12-week group (*p* < 0.05). TT3 and FT3 levels significantly decreased in the 4-week group. TPOAb and TRAb levels decreased after treatment in both groups (*p* < 0.001). Moreover, a significant decrease in TGA levels was observed after treatment in the 4-week group (*p* = 0.006).

### Comparison of changes in metabolic indicators after treatment in both groups

There were no significant differences in the changes in HDL, TT3, FT3, TPOAb, OC, PINP, and CTX after treatment (namely, ΔHDL, ΔTT3, ΔFT3, ΔTPOAb, ΔOC, ΔPINP, and ΔCTX) between the two groups (Table 2). In contrast, the patients in the 12-week group had a greater change in TRAb value (namely, ΔTRAb) than those in the 4-week group (*p* = 0.036).

### Immunologic index measurements

ΔCAS-max represents the changes in the CAS values after treatment. According to the ΔCAS-max (≥3 or <3), we divided the patients into two subgroups (4-week and 12-week groups). The CD16/CD56+ natural killer (NK) cell levels significantly increased only in the 12-week group, whereas IgG and IgE decreased in both the ΔCAS-max greater than or

**TABLE 2** Comparison of changes in metabolic indicators after treatment in both groups

	12 week	4 week	<i>p</i> value
ΔHDL <sup>a</sup>	-0.41 ± 0.28	0.42 ± 0.29	0.928
ΔTT3 <sup>c</sup>	0.17 (0.02,0.54)	0.31 (0.05,0.45)	0.688
ΔFT3 <sup>c</sup>	0.49 (0.17,1.07)	0.59 (-0.10,1.23)	0.947
ΔTPOAb <sup>b</sup>	16.8 (0.88,131.45)	0.70 (0.10,48.55)	0.072
ΔTRAb <sup>b</sup>	8.19 (1.93,18.81)	3.45 (0.83,5.54)	0.036
ΔOC <sup>b</sup>	4.42 (-1.49,11.81)	10.55 (-0.51,14.73)	0.853
ΔPINP <sup>a</sup>	48.93 ± 47.75	34.535 ± 42.26	0.374
ΔCTX <sup>b</sup>	0.37 (0.07,0.97)	0.11 (-0.03,0.21)	0.146

Δ: The value before treatment minus the value after treatment, it represents the change of the value after treatment; CTX, carboxyterminal telopeptide of type I collagen; HDL, high-density lipoprotein; OC, osteocalcin; PINP, aminoterminal propeptide of type I collagen; TPOAb, thyroid peroxidase antibody.

<sup>a</sup>T-test.

<sup>b</sup>Log10 normal transformed before *t*-test.

<sup>c</sup>Mann-Whitney *U* test.

equal to 3 and ΔCAS-max less than 3 subgroups after therapy (*p* < 0.05; Table 3). Moreover, the numbers of CD3+ T cells, CD4+/CD8+ T cells, and IgA decreased after treatment in patients in the 12-week group with a ΔCAS-max less than 3 (*p* < 0.05). The number of CD19+ B cells increased after therapy in the patients in the 4-week group with a ΔCAS-max greater than or equal to 3 (*p* = 0.024), whereas these changes in CD19+ B cells were not observed in patients in the 4-week group with a ΔCAS-max less than 3. The patients in the 4-week group had lower IgG and IgA levels after treatment (Table 3).

**TABLE 3** Comparison of immunological indexes before and after treatment in both groups

	Baseline	After treatment	T value	p value
12-week & ΔCAS-max ≥ 3				
CD3+	67.65 ± 8.08	62.76 ± 6.59	1.966	0.081
CD3+CD4+	42.73 ± 9.38	36.63 ± 6.93	2.219	0.054
CD3+CD8+	23.74 ± 7.92	23.91 ± 9.15	-0.197	0.848
CD4+/CD8+	2.12 ± 1.26	1.92 ± 1.20	2.044	0.071
CD19+	19.05 ± 6.88	17.78 ± 6.26	0.585	0.573
CD16/CD56+	12.51 ± 7.73	18.88 ± 8.01	-2.944	0.016
IgG	11.66 ± 6.16	8.62 ± 3.09	2.401	0.047
IgM	0.98 (0.79,1.66)	1.04 (0.75,1.50)	-0.404	0.698
IgA	2.23 ± 0.84	2.05 ± 0.87	1.345	0.221
IgE	44.70 (24.85,109.50)	32.50 (18.50,76.95)	3.836	0.006
12-week & ΔCAS-max <3				
CD3+	69.77 ± 7.41	62.38 ± 15.01	2.436	0.035
CD3+CD4+	45.62 ± 5.71	41.15 ± 14.68	1.027	0.326
CD3+CD8+	22.62 ± 7.13	25.03 ± 11.41	-1.387	0.193
CD4+/CD8+	2.24 ± 0.85	1.83 ± 0.81	2.452	0.032
CD19+	16.68 ± 5.50	16.54 ± 6.59	0.101	0.921
CD16/CD56+	11.19 ± 6.50	19.01 ± 13.85	-2.229	0.048
IgG	9.89 ± 1.32	8.03 ± 1.39	8.037	< 0.001
IgM	1.14 (0.84,1.71)	1.10 (0.84,1.76)	-0.034	0.973
IgA	2.16 ± 1.17	1.74 ± 0.91	4.494	0.001
IgE	58.50 (36.15,126.00)	20.60 (17.70,57.80)	4.815	0.001
4-week & ΔCAS-max ≥ 3				
CD3+	61.50 ± 9.70	54.00 ± 13.80	2.152	0.068
CD3+CD4+	36.38 ± 9.21	30.38 ± 8.83	1.673	0.138
CD3+CD8+	21.63 ± 6.76	21.25 ± 6.36	0.226	0.827
CD4+/CD8+	1.92 ± 1.04	1.50 ± 0.43	1.165	0.282
CD19+	19.00 ± 5.18	25.75 ± 7.91	-2.871	0.024
CD16/CD56+	17.63 ± 10.78	19.38 ± 8.31	-0.489	0.64
IgG	10.80 ± 1.86	8.08 ± 1.77	4.604	0.002
IgM	1.04 (0.67,1.65)	0.98 (0.72,1.27)	0.563	0.589
IgA	2.42 ± 0.59	1.83 ± 0.56	4.409	0.002
IgE	22.25 (18.20,64.60)	47.05 (18.08,85.80)	1.597	0.149
4-week & ΔCAS-max <3				
CD3+	75.14 ± 7.21	65.47 ± 14.35	2.078	0.129
CD3+CD4+	32.29 ± 3.90	30.78 ± 13.73	0.192	0.86
CD3+CD8+	40.88 ± 6.87	32.52 ± 9.52	2.141	0.122
CD4+/CD8+	0.66 ± 0.40	1.04 ± 0.64	-0.72	0.524
CD19+	10.78 ± 2.52	17.57 ± 5.85	-1.96	0.145
CD16/CD56+	12.86 ± 5.05	15.20 ± 13.38	-0.414	0.707
IgG	11.79 ± 1.58	8.46 ± 1.86	5.355	0.003
IgM	1.24 (1.10,1.88)	1.63 (0.67,1.66)	1.387	0.224
IgA	2.22 ± 0.44	1.76 ± 0.56	3.9	0.011
IgE	17.70 (17.20,98.90)	28.85 (17.58,100.33)	0.833	0.452

ΔCAS-max: CAS before treatment minus after treatment, and take the maximum value of the left and right eyes.



	12-week	4-week	T value	p value
$\Delta$ CD3+	6.10 $\pm$ 8.74	9.36 $\pm$ 10.15	-1.026	0.312
$\Delta$ CD3+CD4+	5.16 $\pm$ 12.05	6.65 $\pm$ 13.91	-0.343	0.734
$\Delta$ CD3+CD8+	-1.29 $\pm$ 4.75	2.44 $\pm$ 6.60	-1.995	0.054
$\Delta$ CD4+/CD8+	0.31 $\pm$ 0.47	0.50 $\pm$ 1.70	-0.419	0.681
$\Delta$ CD19+	0.80 $\pm$ 5.67	-6.21 $\pm$ 6.12	3.538	0.001
$\Delta$ CD16/CD56+	-7.20 $\pm$ 9.68	-3.23 $\pm$ 11.03	-1.149	0.259
$\Delta$ IgG	2.36 $\pm$ 2.39	2.68 $\pm$ 1.68	-0.479	0.635
$\Delta$ IgM	-0.04 $\pm$ 0.18	0.05 $\pm$ 0.21	-1.454	0.155
$\Delta$ IgA	0.32 $\pm$ 0.35	0.51 $\pm$ 0.36	-1.654	0.107
$\Delta$ IgE	26.50 (7.10,70.2)	2.20 (0,11.35)	2.816	0.009

$\Delta$ : The value before treatment minus the value after treatment, it represents the change of the value after treatment.

	Week (n = 17)	Week (n = 11)	T	p value
$\Delta$ ADC-max (right eye)	273.64 $\pm$ 170.07	382.87 $\pm$ 195.79	-1.565	0.13
$\Delta$ ADC-max (left eye)	286.14 $\pm$ 176.73	384.15 $\pm$ 229.65	-1.274	0.214
$\Delta$ ADC-max	325.24 $\pm$ 153.76	497.80 $\pm$ 191.92	-2.632	0.014
$\Delta$ CAS (right eye) <sup>a</sup>	2 (2, 4)	3 (2, 4)	/	0.722
$\Delta$ CAS (left eye) <sup>a</sup>	2 (1, 3.5)	2.5 (1, 3)	/	0.610
$\Delta$ CAS-max <sup>a</sup>	3 (2, 4)	3 (2, 4)	/	0.629

Abbreviations: ADC, apparent diffusion coefficient;  $\Delta$ ADC-max (right eye), ADC before treatment minus after treatment, and take the maximum value of  $\Delta$ ADC of 4 eye muscles of right eye;  $\Delta$ ADC-max (left eye), ADC before treatment minus after treatment, and take the maximum value of  $\Delta$ ADC of 4 eye muscles of left eye;  $\Delta$ ADC-max, ADC before treatment minus after treatment, and take the maximum value of  $\Delta$ ADC of 8 eye muscles;  $\Delta$ CAS-max, CAS before treatment minus after treatment, and take the maximum value of the left and right eyes.

<sup>a</sup>Mann-Whitney *U* test.

There were no significant changes in the CD series or IgGs between the  $\Delta$ CAS-max greater than or equal to 3 and  $\Delta$ CAS-max less than 3 subgroups after therapy (Table S1).

The patients in the 4-week group had a greater change in CD19+ B cells ( $\Delta$ CD19+) than those in the 12-week group ( $p = 0.001$ ; Table 4). However, the patients in the 12-week group had a greater change in IgE ( $\Delta$ IgE) levels than those in the 4-week group ( $p = 0.009$ ; Table 4).

### Comparison of changes in the ADC and CAS values after treatment in both groups

Seventeen patients in the 12-week group and 11 patients in the 4-week group had available ADC data. Table 5 compares the changes in the ADC and CAS values in these patients before and after treatment. As shown in Table 5, the change in the ADC values, namely, the  $\Delta$ ADC-max (the ADC before treatment minus the ADC after treatment, taking the maximum value of the  $\Delta$ ADC from 8 eye muscles), was greater in the 4-week group than in the 12-week group ( $p = 0.014$ ). This result indicated that the decrease in the ADC value in

**TABLE 4** Comparison of changes of value in immunological indexes after treatment in both protocol

**TABLE 5** Comparison of changes of value in ADC and CAS after treatment in both protocols

the 4-week group was more significant than that in the 12-week group. The difference in  $\Delta$ CAS-max was not significant between the two groups (Table 5).

## DISCUSSION

TAO, also known as GO, is an extrathyroidal manifestation of GD. Many risk factors promote the development of GO, such as tobacco exposure, uncontrolled thyroid function, oxidative stress, and a high level of TRAb.<sup>17,18</sup> It has been estimated that 1 in 20 patients with GD had moderate-to-severe GO.<sup>19</sup> Methylprednisolone monotherapy leads to satisfactory outcomes in most patients with active and moderate-to-severe TAO.<sup>20</sup> Moreover, IVGC plus orbital radiotherapy or mycophenolate mofetil (MMF) has also improved symptoms in patients with active and moderate-to-severe TAO.<sup>20,21</sup> Although higher glucocorticoid (GC) doses have been associated with slightly improved response rates, the frequency of serious adverse events rose to unacceptable levels. Therefore, due to safety concerns, cumulative doses of methylprednisolone of less than 8 g are recommended.

However, the metabolic outcomes of IVGC have not been comprehensively assessed in these studies.

In previous studies, a limited number of patients in the 4-week group discontinued GC therapy due to adverse reactions, such as impaired liver function or severe gastrointestinal symptoms during treatment. For example, in the study of Zhu et al.,<sup>10</sup> one patient out of 41 showed impaired liver function during the 4-week GC therapy. One patient in the 4-week group developed intractable hiccups in the third week of treatment. However, in our study, no patients discontinued treatment due to adverse reactions, such as severe liver damage or severe gastrointestinal symptoms caused by GC treatment.

In our study, we found that metabolic disorders in glucose and lipids (FPG, HbA1c, TC, and LDL) were more obvious in the 12-week group than 4-week group, and the 4-week group did not have a higher incidence of severely impaired liver function, gastrointestinal symptoms, or other adverse events than the 12-week group. Previously, alterations in glucose and lipids were seen as adverse events in patients with TAO treated with IVGC. Zhu et al.<sup>10</sup> found no significant difference in weight gain, bone mineral density, or glucose and lipid levels between the 4-week and 12-week protocols. The reasons why our findings differ from Zhu et al. are as follows. First, the differences may be related to the inconsistency of the observation time in these two studies. In their study, the effects of GCs on glucose and lipid metabolism were observed in the 4-week and 12-week groups in the 12th week. In our study, the effects of GCs on glucose and lipid metabolism were observed in the 4th week in the 4-week group and in the 12th week in the 12-week group. However, both studies lacked a long-term follow-up to observe the effects of GCs on glucose and lipid metabolism. Second, GCs can regulate glucose homeostasis by promoting gluconeogenesis in the liver and decreasing glucose uptake and utilization in skeletal muscle and white adipose tissue. A study showed that the increase in insulin resistance and the decrease in insulin secretion did not occur after 3 days of prednisone treatment especially in healthy subjects.<sup>22</sup> In addition, acute  $\beta$ -cell damage caused by GCs has been shown to be temporary and reversible.<sup>23</sup> Thus, short-term GC exposure may have less effect on glucose tolerance, whereas long-term GC exposure resulted in hyperglycemia and insulin resistance.<sup>24,25</sup>

GC-induced osteoporosis is the primary cause of secondary osteoporosis. GCs cannot only induce the differentiation of osteoblasts to adipocytes and the apoptosis of mature osteoblasts, but also inhibit the synthesis and secretion of bone matrix proteins, growth factors, and cytokines.<sup>26</sup> Bone turnover markers are the products of bone tissue metabolism and are divided into bone formation markers and bone resorption markers. The former reflects osteoblast activity and the bone

formation status, and the latter represents osteoclast activity and the bone resorption level. Among these markers, ALP, OC, and PINP are markers of bone formation, and CTX is a marker of bone resorption. The analysis of these markers can help determine the type of bone turnover, predict the rate of bone loss, assess the risk of fracture, and understand the progress of the disease. Shen et al. studied the effect of GC withdrawal on bone impairment in a rat model. The results indicated that CTX and PINP were higher in the GC group than in the control group 3 months after GC withdrawal.<sup>26</sup> In patients with multiple sclerosis, methylprednisolone (15 mg/kg/d) was infused for 10 days. Dovic et al. found that OC and PINP dramatically decreased and persisted throughout the whole treatment period. ALP levels showed only a modest decrease on day 6. CTX levels showed a progressive increase during treatment.<sup>27</sup> Moreover, consistent with animal experiments, OC, PINP, and ALP increased 3 months after GC withdrawal. In our study, bone metabolic markers decreased in both the 4-week and 12-week groups. However, these two treatments had similar influences on bone metabolism. Based on previous studies, we assumed that the earlier the IVGC was withdrawn, the sooner the bone metabolic indexes recovered.

Adverse events with IVGC in the cardiovascular and cerebrovascular systems and liver have been reported and associated with a high single (more than 0.5 g) and/or cumulative dose (more than 8 g).<sup>13,28</sup> In a large retrospective cohort study, Sisti et al. found that age (over 53 years) and GC dose (single dose more than 0.57 g and/or cumulative dose more than 8.5 g) play important roles as risk factors for acute liver damage.<sup>29</sup> In our study, we did not observe a significant elevation in ALT or AST, which indicated that IVGC therapy was safe in both groups.

The relationship between TRAb and TAO is relatively complicated. Seo et al. indicated that thyrotropin-binding inhibitory immunoglobulin levels, rather than TRAb levels, were closely associated with the onset and severity of TAO.<sup>30</sup> In another study, treatment with IVGC plus MMF for 36 weeks showed no advantage in alterations in TRAb levels compared with MMF. However, the IVGC plus MMF group had an improved rate of response to therapy by 24 weeks in patients with active and moderate-to-severe GO.<sup>20</sup> Therefore, alterations in TRAb did not coincide with changes in symptoms. We found that TRAb was decreased in both the 4-week and 12-week groups. However, TRAb declined more sharply in the 12-week group. At present, there is still much controversy about whether TGAb is involved in the pathogenesis of TAO, and the correlation between TGAb and the severity of TAO has not been well verified. We found a decrease in TGAb after treatment in both groups, but the decrease in TGAb in the 12-week group was not statistically significant. This may be related to the inconsistent follow-up time of the two treatment regimens. This finding indicated that the effect

of short-term high-dose GC exposure on TGAb is more significant than that of chronic exposure. We speculated that TGAb may be directly or indirectly related to the pathogenesis of TAO, which could provide a perspective for studying the pathogenesis of TAO.

The TSH receptor is expressed on orbital fibroblasts and may be a target for the IgG-stimulated hyperproduction of adipose tissue, and both Th1 and Th2 cytokine patterns are involved in this immunization.<sup>31</sup> A recent study helped to explain the role of Th17 cell immunity in TAO, and the authors proposed that although both Th17- and Th2-related elements were associated with TAO development, only the Th17 pathway was positively correlated with CAS and eyesight.<sup>32</sup> They also found that the presence of effector B cells expressing high CD27 and low CD19 in the TAO orbital microenvironment may indicate an early inflammatory stage of TAO, and the CD3+CD8+ T-cell subpopulation was not responsible for TAO inflammation.<sup>32</sup> In our study, the proportion of blood CD19+ B cells in the 4-week group significantly increased after therapy. However, the proportion of CD19+ B cells in the 12-week group did not significantly change after treatment. Some researchers have suggested that autoantibodies may not be produced by peripheral B lymphocytes but may be produced by lymphocytes in the thyroid or in other lymphoid organs.<sup>33</sup> The number of CD19+ B cells in the orbital tissue is low in the early stage of inflammation,<sup>32</sup> and the number of CD19+ B cells in peripheral blood increased after short-term high-dose GC treatment in our study, which indicated that we can further study the level of CD19+ B cells in local orbital tissue after GC treatment in the future. Although the specific pathway is currently unknown, it may provide insight into the pathogenesis and treatment of TAO. We also found that the proportion of NK cells in the 12-week group significantly increased after therapy. Wenzel et al. showed that NK cells had no correlation with the activity and severity of GO.<sup>34</sup> Other studies have shown that methylprednisolone can inhibit prostaglandin secretion, fibroblast activity, and glycosaminoglycan production.<sup>35</sup> Prostaglandins have an inhibitory effect on the activity of NK cells, and methylprednisolone may relieve this inhibition, which may have led to the increase in the proportion of NK cells that was observed in this study.

For a long time, IgE was thought to play an important role in allergies. More than 4 decades ago, IgE was found in patients with GD.<sup>36</sup> Other studies also reported elevated levels of IgE in patients with autoimmune thyroid diseases, especially in those with GD, in different countries.<sup>37–39</sup> Thereafter, Raikow et al. found that IgE was present in various orbital tissues in patients with TAO regardless of the disease severity and site biopsied.<sup>40</sup> In patients with TAO, Th2-type cytokines (IL-4, IL-5, and IL-6) promoted IgG production, including IgE production. There was also a correlation between the total IgE levels and the severity of ophthalmopathy

in GD. Molnár et al. measured IgE levels in patients with GD and healthy controls. The results showed that IgE levels were higher in patients with TAO than in controls. Notably, anti-thyroid and GC treatment decreased total IgE levels.<sup>41</sup> In our study, 12-week treatment dramatically decreased IgE levels in both the  $\Delta$ CAS-max greater than or equal to 3 and  $\Delta$ CAS-max less than 3 subgroups. This finding may suggest that alterations in IgE may not be significantly related to changes in the CAS score. However, IgE levels slightly decreased after 4-week therapy. We hypothesize that short-term, high-dose GC exposure has a limited effect on IgE in peripheral blood. In addition, the baseline IgE level in the 12-week group was higher than that in the 4-week group, which may be one of the reasons why the  $\Delta$ IgE was more significant in the 12-week group than in the 4-week group.

The ADC and signal intensity ratio values are objective MRI parameters that correlate well with CAS values and muscle dysfunction in patients with TAO.<sup>9</sup> Strong T2W and fat-suppressed images obtained using turbo inversion recovery intensity and short-tau inversion recovery sequences have been shown to be useful in distinguishing EOM edema, but they do not effectively detect subsequent changes in inflammation.<sup>42</sup> However, no head-to-head randomized controlled trial has used the ADC of MRI as an objective evaluation index to compare the efficacy of 4-week and 12-week IVGC therapy for treating moderate-to-severe GO. Therefore, in our study, we used the ADC as an indicator of the severity of EOM inflammation in patients with TAO. In our study, the ADC in the 4-week group was more significantly improved than that in the 12-week group, which shows that the improvement in EOM inflammation in the 4-week group was more significant. However, there was no significant difference in CAS values between the two groups, and this finding is consistent with the results of Zhu et al.<sup>10</sup>

Our study has some limitations. First, this is a retrospective study rather than a prospective cohort. Second, the follow-up time was different between the 4-week and 12-week groups. The patients with TAO were evaluated using the same dose of IVGC, and we did not observe the long-term effect of IVGC in the 4-week group. Third, only some patients underwent orbital MRI before and after treatment. Therefore, the use of ADCs in patients with TAO still needs to be examined. Finally, we did not measure other cytokines closely related to TAO, such as IL-4, IL-6, IL-13, and CXCL10; instead, we measured IgG and CD. Furthermore, a prospective study will be conducted to compare the changes in ADC of the EOMs and metabolic index after 4-week or 12-week IVGC therapy with the same follow-up time.

In summary, our study indicated that the ADC was improved after 4-week therapy for active moderate-to-severe TAO. However, there was no significant difference in the changes in CAS after therapy between the two groups.

Metabolic disorders involving glucose and lipids were more obvious in the 12-week group than in the 4-week group. Therefore, 4-week therapy was recommended for patients with TAO with glucose and lipid disorders.

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## CONFLICT OF INTEREST

The authors declared no competing interests for this work.

## AUTHOR CONTRIBUTIONS

X.C. and B.H. wrote the manuscript. Y.L., B.H., and H.Z. designed the research. X.C., B.A., Qin Li, Qing Li, J.Q., D.L., C.S., L.Y., H.Z., B.J., N.W., M.J., X.T., Z.S., C.Z., Y.M., P.X., and J.S. performed the research. X.C., B.A., and Qin Li analyzed the data. X.C., Y.L., B.H., and H.Z. contributed new reagents/analytical tools.

## ETHICAL APPROVAL

The study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the appropriate institutional review committee.

## DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available on reasonable request from the corresponding authors.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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