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Effect of calorie restriction on thyroid antibodies and symptoms in Hashimoto's thyroiditis patients: a randomized controlled trial

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ABSTRACT: Hashimoto's thyroiditis (HT) is an autoimmune disease, characterized by abnormal elevation in thyroid peroxidase antibody (TPO-Ab) and/or thyroglobulin antibody (TG-Ab). The antibody titers are positively correlated with multiple symptoms despite adequate hormone substitution. However, no specific treatment exists to reduce the levels of antibodies. We carried out a randomized controlled trial to determine the effect of calorie-restricted (CR) diet on Hashimoto disease. Results showed that serum TPO-Ab (-191.46 KU/L, 95% CI: -278.35 to -104.58) and TG-Ab (-49.30 KU/L, 95% CI: -99.12 to 0.53) in the CR group were sharply reduced, accompanied by an improvement in thyroid gland shown by magnetic resonance imaging. The CR group exhibited significant alleviation of non-hypothyroid symptoms, with this improvement positively correlating with the reduction of thyroid antibodies. Moreover, CR also improved immune dysfunction, as well as decreased levels of pro-inflammatory biomarkers and serum lipids. These findings provide evidence that calorie restriction may serve as a potential adjuvant treatment strategy for patients with HT.

Keywords: Hashimoto's thyroiditis; Calorie restriction; Thyroid antibody; Symptoms; Immune dysfunction; Inflammation

1. Introduction

Hashimoto's thyroiditis (HT) is an autoimmune disorder that damages the thyroid gland, a small, butterfly-shaped gland located in the neck [1]. In recent years, HT has become one of the most common thyroid diseases, affecting about 1%~2% of people worldwide [2]. Women are more susceptible than men, with a female-to-male ratio of 7-10:1 [3]. In this disorder, the body's self-immune system mistakenly attacks the thyroid gland and the gland undergoes an inflammatory response and damage, ultimately leading to irreversible hypothyroidism [4-6]. Thyroid autoantibodies are key markers in the diagnosis of HT. Thyroid peroxidase antibody (TPO-Ab) is detected in approximately 90%-95% of patients, while thyroglobulin antibody (TG-Ab) is present in 60%-80% [7]. Nevertheless, a minority of patients may present with normal antibody levels, a condition often referred to as seronegative HT, in which diagnosis relies on

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ultrasonographic or histopathological features [3]. Current treatments are only available for patients who has progressed to hypothyroidism, typically involving hormone replacement therapy, which aims to restore normal levels of thyroid hormone [8]. However, there remains no effective cure to reverse the immune dysfunction, by decreasing the elevated levels of TG-Ab or TPO-Ab [9]. Even though thyroid hormones are kept within normal range by medicine, the patients still have multiple complications, such as abdominal distension, weight gain, forgetfulness, anxiety, fatigue, insomnia and rash [9-11]. It is therefore necessary to find effective interventions to improve immune system and symptoms of HT patients.

During the last decades, epidemiological studies have suggested that a healthy diet is critical for the prevention of autoimmune diseases, and calorie restriction (CR) is one of the most intensively studied nutritional intervention closely related to health benefits [12-15]. CR diet has been shown to extend lifespan, promote weight loss, improve metabolic health, and prevent neurodegenerative diseases [16-20]. Recent studies on human and animals have suggested that CR can improve immune dysfunction [21-23]. In addition, CR has been linked to a powerful anti-inflammatory effect in humans. It can reduce the levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-12 (IL-12), and increase the hormones that can suppress inflammation, including cortisol, adiponectin and ghrelin [24-27].

Given the benefits of CR diet for immune dysfunction and reducing inflammation, we hypothesized that CR may protect against the progression of HT. In the present study, a 12-week randomized controlled trial (RCT) was conducted to primarily investigate if CR diet could decrease the levels of TPO-Ab and TG-Ab in HT patients. Secondly, this study also aimed to evaluate the positive effect of CR on immune dysfunction, inflammation, thyroid function, and non-hypothyroid symptoms specifically related to HT.

2. Materials and Methods

2.1 Study design

This was a 12-week RCT study, conducted at the Second Affiliated Hospital of Zhejiang Chinese Medical University. The trial was registered at chictr.org.cn (ChiCTR2200060968). The protocol was approved by the Ethics Committee of Medical ethics at Zhejiang Chinese Medical University (No. 20220607-2) and has been published elsewhere [28]. Written informed consent was provided by each participant.

2.2 Study Participants

All participants were HT patients living in Hangzhou, China. They were recruited via posters, leaflets and advertisements on social media during July 2022 to September 2022. The main inclusion criteria were: age between 18-65 years old; previously diagnosed as HT by an endocrinologist according to the Guidelines for the Diagnosis and Treatment of Thyroid Diseases in China; had a baseline TPO-Ab level above 27 KU/L or TG-Ab above 12 KU/L (three times the upper limit of normal, ULN). This threshold was set to minimize the inclusion of borderline positive cases and to ensure that participants had clear evidence of thyroid autoimmunity. In addition, individuals with borderline antibody levels may have less motivation or

compliance during follow-up interventions, which could potentially affect study quality. Key exclusion criteria included (1) history of thyroid trauma or surgical treatment; (2) with other autoimmune diseases, such as systemic lupus erythematosus or rheumatoid arthritis; (3) with serious chronic diseases, such as cancer, heart disease, hepatic or renal dysfunction; (4) pregnancy or lactation period; and (5) participation in another clinical trial within three months.

2.3 Procedures

A total of 266 potential adult participants with HT disease were initially recruited for the study. Subsequently, they underwent meticulous screening in accordance with the inclusion and exclusion criteria. Finally, 66 eligible participants were enrolled and randomized into either a calorie-restricted group (CR) or a calorie-unrestricted control group (Control). Randomization was block-stratified by age, gender and thyroid antibody of the subjects to avoid potential difference between two groups at baseline, using a computer-generated random number sequence prepared by a statistician with no clinical involvement in this trial.

The intervention lasted for 12 weeks, all the participants were instructed to consume a diet that included a combination of 45%-55% calories from carbohydrates, 20%-30% from fats, and 15%-25% from proteins. The macronutrient distribution was chosen to enhance satiety and adherence, while addressing potential lipid metabolism abnormalities that are common in HT patients. Participants in CR group were required to limit their calories intake equal to their basal energy expenditure (BEE), which was measured by a body composition analyzer at baseline. It meant that daily caloric intake in CR group was limited by 20%-30%, as the BEE accounts for approximately 70%-80% of the total daily calorie requirement.

Dietary counseling was conducted by trained dietitians. All the participants received a seven-day meal plan and several sample menus weekly through WeChat. Participants were encouraged to weigh and record foods to ensure they accurately reported their caloric intake, and they were required to note and take photo of the food they eat and send to the dietitian every day. With the note and photograph of the food, two researchers assessed the energy intake of each participant via Nutrition Calculator version 11.0 (Qingdao University, China). The trained dietitian and researchers discussed the problems of the participants' diet together on each Saturday and provided them reasonable suggestions as feedback. The subjects also received telephone calls weekly and met with the dietitian every two weeks to aid their adherence to the project and to ensure adequate nutrient intake. Besides, all participants were encouraged to maintain their original exercise habits throughout the intervention period. More detailed procedures and method were shown on the supplementary materials and protocol [28].

2.4 Outcomes

The primary outcome was the between-group difference of the changes in thyroid antibody levels (TPO-Ab and TG-Ab) from baseline to 12 weeks. Secondary outcomes included mean changes in thyroid function indexes (thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4)), morphology parameters of thyroid (T1-weighted images (T1WI), T2-weighted images (T2WI), apparent

diffusion coefficient (ADC)), T lymphocyte subpopulations, inflammatory biomarkers, lipid metabolism and self-reported symptoms related to HT from baseline to 12 weeks.

2.5 Blood analysis

Serum and plasma were separated from blood samples after centrifugation with 3,000 rpm at 4°C for 10 min, and immediately stored at -80°C until use. Serum concentrations of thyroid parameters (TPO-Ab, TG-Ab, TSH, FT3, FT4) were measured by direct chemiluminescence using acridinium ester technology (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics, Munich, Germany). Plasma inflammatory biomarker concentrations, including IL-2, IL-4, IL-6, IL-10, IL-17A, interferon-γ (IFN-γ), and TNF-α, were detected with ELISA kits (BD Biosciences, San Jose, CA, USA). T lymphocyte subpopulations were measured with blood samples by a flow cytometry (BD, USA). Lipids profiles (total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)) in serum were measured with an automatic analyzer (HITACHI 7020, Sysmex Kobe, Japan). Laboratory assays were performed in duplicate to minimize analytical errors, and all measurements were performed at the end of the study to minimize variability.

2.6 Thyroid magnetic resonance imaging (MRI)

Thyroid MRI examinations were performed on the enrolled participants at baseline and weeks 12. Participants were advised to breathe slowly and steadily, and not to swallow or speak during the scanning process to reduce motion artifacts. T1WI was performed using the following parameters: time of repetition (TR), 524ms; time of echo (TE), 11ms; excitation frequency, 2; field of view (FOV), 220mm × 220mm; slice thickness, 4mm; slice gap, 1.2 mm; and 20 scanning layers. T2WI was performed using the following parameters: TR, 2410ms; TE, 75ms; excitation frequency, 2; FOV, 220mm × 220mm; slice thickness, 4 mm; slice gap, 1.2 mm; and 20 scanning layers. ADC value was obtained as follows: Select the largest section of both thyroid lobes, draw region of interest along the edge of the thyroid parenchyma, and take the average of three consecutive measurements as the ADC value of one thyroid lobe. Then take the average of both thyroid lobes as the ADC value of the thyroid. All the values of the thyroid parenchyma of HT patients were measured by two radiologists, three times in each case, with the same area as far as possible in each measurement, and the average of the three measurements was taken as the value of the site. Through the above measurements, we obtained T1WI, T2WI, ADC values to evaluate the changes of thyroid.

2.7 General Demographic Information Questionnaire

General Demographic Information Questionnaire was filled at baseline. The questionnaire including name, gender, age, contact number, home address, marital status, education level, occupation, economic income, smoking history (whether and how much you smoke), alcohol history (whether you drink alcohol and the type and number of alcohol consumed), physical activity, disease history (whether there is hypertension, diabetes, coronary heart disease, hyperlipidemia, etc.), family genetic history (whether the subject's immediate family members have a history of Hashimoto's thyroiditis, hyperthyroidism, Graves' disease, idiopathic

myxedema and other diseases), and drug use (information on whether you have taken drugs to improve thyroid function, hepatoprotective drugs, antihypertensive drugs, hypoglycemic drugs, lipid-lowering drugs, etc.), and the use of dietary supplements.

2.8 Symptom analysis and scoring criteria of Hashimoto's Thyroiditis Symptom Questionnaire

We used the Hashimoto's Thyroiditis Symptom Questionnaire to assess the symptoms related to Hashimoto disease at both baseline and weeks 12. The questionnaire consists of 49 items summarized in 8 domains, including digestive system (8 symptom items), respiratory system (2 symptom items), circulatory system (4 symptom items), endocrine system (6 symptom items), neuropsychiatric system (11 symptom items), movement system (4 symptom items), reproductive system (4 symptom items), and mucocutaneous system (10 symptom items). Some of these symptoms were specifically relevant to thyroid diseases (e.g., symptoms of hyperthyroidism and goiter), while some were not, but frequently reported by HT patients (e.g., fatigue, intestinal problems). Responses for each item will be scored according to symptoms severity with levels 1-10, where 1 means no symptoms, 10 means symptoms seriously affect lifestyle.

2.9 Statistical analysis

Main data were analyzed according to the intention-to-treat principle. Exploratory data analysis and Shapiro-Wilk tests were conducted to assess the normality of the data distribution. Continuous variables were described as means ± standard deviation (SD) when they are normal distributed, and as median and inter-quartile range (IQR) if not. Between-group difference at baseline and in the change from baseline to the middle or end of the intervention were analyzed with unpaired *t* test when data were normal distribution, while with Mann-Whitney U tests for abnormal distribution data [29]. For categorical variables, data were expressed as counts and percentages. Between-group comparisons of categorical variables were determined with the chi-square test. The correlation between the decrease of thyroid antibodies and the improvement of symptom and inflammation was analyzed using Spearman's correlation test. The level of significance was set as a two-sided *P* value less than 0.05.

The power calculation was based on a between group difference in TPO-Ab changes of 100 KU/L with a SD of 150 KU/L, referred to a previous study and considered to be clinically meaningful [30]. We required at least 52 individuals to complete the trial to achieve a power of 80% for a two-side test with significant level of 0.05. Considering a 20% chance of dropping out, the sample size was increased to 65 patients. All statistical analyses were performed with SPSS 27.0.

3. Results

3.1 Population characteristics and compliance of the participants

From the 266 recruited HT patients, 66 patients met the enrollment criteria (n = 33 in each group). The mean (± SD) age of the participants was 32.5 ± 10 years, and 94% of them were female. At baseline, characteristics and physical activity of the participants were similar between the two groups (Table 1, Table S1). During the intervention, three participants in CR group and five participants in control group terminated

the trial prematurely, with retention rates of 90.91% and 84.85%, respectively (Figure 1). The percentage of days on which participants adhered to the prescribed calories intake was 89.9% ± 3.6% in the CR group and 90.9% ± 4.0% in the control group. The mean daily energy intake in the CR group was reduced to a level close to their BEE, and macronutrient distribution generally met recommended ranges (Table S2). Body weight, body fat mass and abdominal visceral fat area were all significantly decreased in the CR group, compared with the control group, indicating a good compliance of the participants (Table S3). However, the intake of several micronutrients, including calcium and vitamin A, was slightly below the corresponding reference nutrient intake (RNI) (Table S4). No adverse events or complications were observed during the study period.

Table 1. Characteristics of the participants at baseline

Variables	Calorie Restriction Group	Control Group	*P* Value
Female, n (%)	31 (93.9)	31 (93.9)	1.000
Age, years	33 ± 11	32 ± 9	0.539
BMI, kg/m^2	22.04 ± 3.10	21.65 ± 2.82	0.601
BEE, kcal	1241.70 ± 94.22	1257.17 ± 123.41	0.573
Smoking, n (%)			
Frequent	0	0	1.000
Occasional/never	33	33	
Drinking, n (%)			
Frequent	5	6	0.741
Occasional/never	28	27	
Family history of HT, n (%)			
Yes	4	4	1.000
No	29	29	
Levothyroxine, n (%)			
Drug-free	29 (87.88)	28 (84.85)	
25 ug/d	0 (0)	1 (3.03)	0.755
50 ug/d	4 (12.12)	4 (12.12)	
TSH, mIU/L	2.67 (2.06, 4.26)	2.48 (1.54, 3.77)	0.297
FT3, pmol/L	4.26 ± 0.38	4.31 ± 0.51	0.644
FT4, pmol/L	14.43 ± 1.72	15.06 ± 2.05	0.178
TPO-Ab, KU/L	277.67 (49.76, 570.35)	152.51 (14.56, 656.12)	0.517
TG-Ab, KU/L	11.03 (1.79, 81.45)	25.07 (3.10, 152.02)	0.459
TC, mmol/L	5.07 ± 0.81	4.64 ± 0.83	0.038
TG, mmol/L	0.80 (0.57, 1.04)	0.81 (0.64, 1.46)	0.206
LDL-C, mmol/L	2.95 ± 0.75	2.73 ± 0.70	0.208
HDL-C, mmol/L	1.72 ± 0.33	1.52 ± 0.39	0.031
$CD3^+$/total lym, %	70 (63.5, 74)	74 (68,77)	0.022
$CD4^+$/total lym, %	32.82 ± 6.31	37.73 ± 6.17	0.002
$CD8^+$/total lym, %	27.48 ± 7.20	28.55 ± 7.13	0.550
$CD4^+/CD8^+$	1.28 ± 0.42	1.45 ± 0.60	0.196
IL-17A, pg/mL	0.66 ± 0.66	0.55 ± 0.67	0.488
IL-4, pg/mL	0.46 ± 0.28	0.46 ± 0.30	0.976
IL-6, pg/mL	1.41 ± 0.44	1.40 ± 1.14	0.957
IL-10, pg/mL	1.57 ± 0.36	1.80 ± 1.04	0.253
TNF-α, pg/mL	1.20 ± 0.45	1.29 ± 0.71	0.504
IFN-γ, pg/mL	2.08 ± 0.51	2.06 ± 0.51	0.853

Data are presented as counts and percentages for categorical variables, as means and standard deviations for normally distributed variables, and as medians and interquartile ranges for non-normally distributed variables. Abbreviations: BMI, body mass index; BEE, basal energy expenditure; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; TPO-Ab, antithyroid peroxidase; TG-Ab, antithyroid thyroglobulin; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; IL, interleukin.

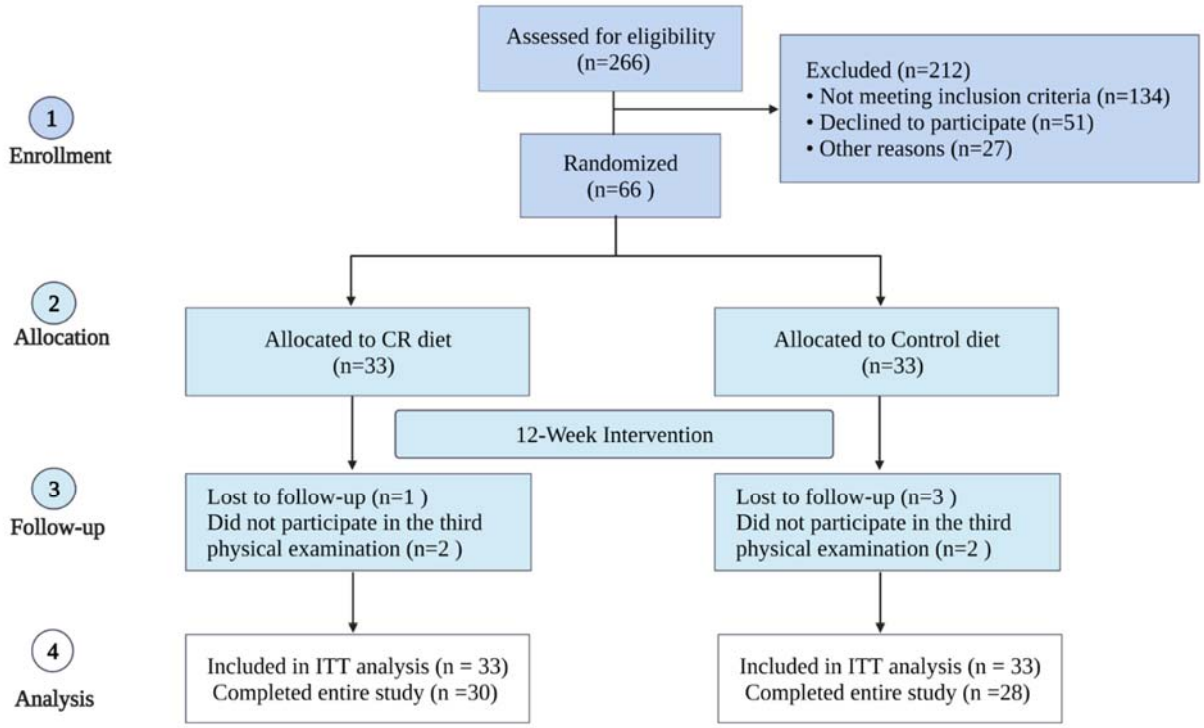

Figure 1. Flow diagram of participants. Participants were randomized into CR group or control group. Abbreviations: CR, calorie restricted; ITT: intention-to-treat.

3.2 Effects of CR diet on thyroid antibody levels

TPO-Ab levels in the CR group decreased by -82.99 KU/L (95% confidence interval [CI]: -138.89 to -27.10) at 6-week, and -191.46 KU/L (95% CI: -278.35 to -104.58) at 12-week, both with a significant between-group difference (Figure 2A-C). TPO-Ab decreasing by more than 100 KU/L accounted for 40% of participants in the CR group and 0% in the control group (Figure 2G). Similarly, TG-Ab levels in the CR group decreased by -51.06 KU/L (95% CI: -97.50 to -4.63) at 6-week, and were -49.30 KU/L (95% CI: -99.12 to 0.53) at 12-week (Figure 2D-F). The change of TG-Ab from baseline to 6-week showed a significant difference with that in control group. TG-Ab decreasing by more than 100 KU/L accounted for 10% of participants in the CR group and 4% in the control group (Figure 2H).

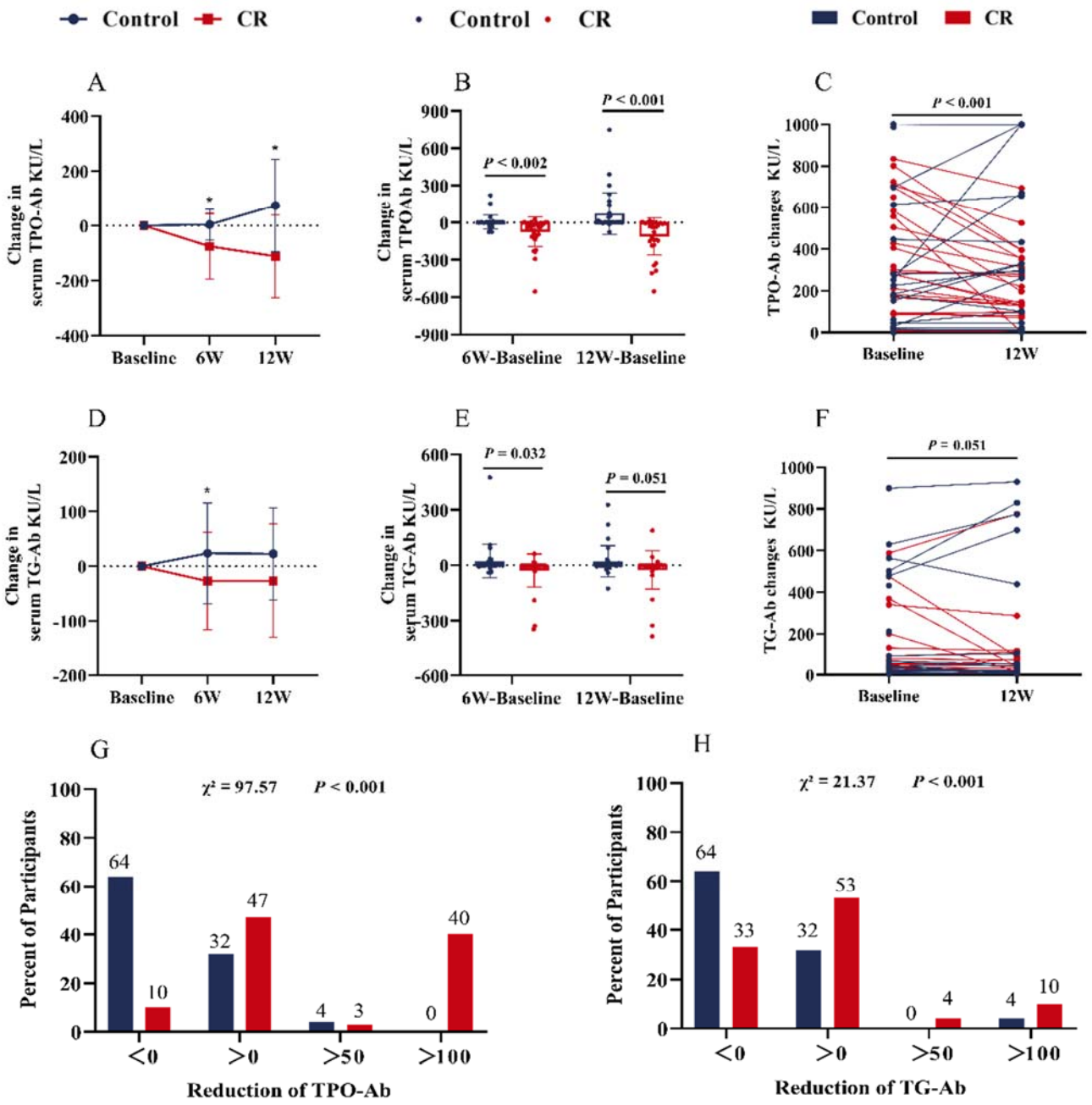

Figure 2. Effect of CR on thyroid antibody levels. Changes of TPO-Ab (A-C) and TG-Ab (D-F) levels from baseline to the middle and the end of intervention in the CR and control groups. Percentage of participants with varying degrees of changes in TPO-Ab (G) and TG-Ab (H) from baseline to 12 weeks. Between-group significance was analyzed by Mann-Whitney U tests. The differences in distribution of participants between groups were analyzed using Fisher's exact test. *Statistically significant mean difference in change between groups, with a $P < 0.05$. Abbreviations: TPO-Ab, thyroid peroxidase antibody; TG-Ab, thyroglobulin antibody; CR, calorie restricted.

3.3 Effects of CR diet on function and morphological assessment of thyroid

No significant differences were observed for changes of TSH, FT3 and FT4 levels between the CR and control groups (Figure 3A-C). However, results from MRI showed that the changes of T1WI and T2WI signal intensity from baseline to 12 weeks were significantly lower in the CR group than that in the control group, indicating that the thyroid was improved after CR diet for 12 weeks (Figure 3D-E). ADC value was also decreased in the CR group, but the difference did not reach a significant level (Figure 3F). These changes were

in accordance with the changes in thyroid morphology, which showed that texture of thyroid become more even and more closed to a healthy thyroid after CR diet for 12 weeks (Figure 3G).

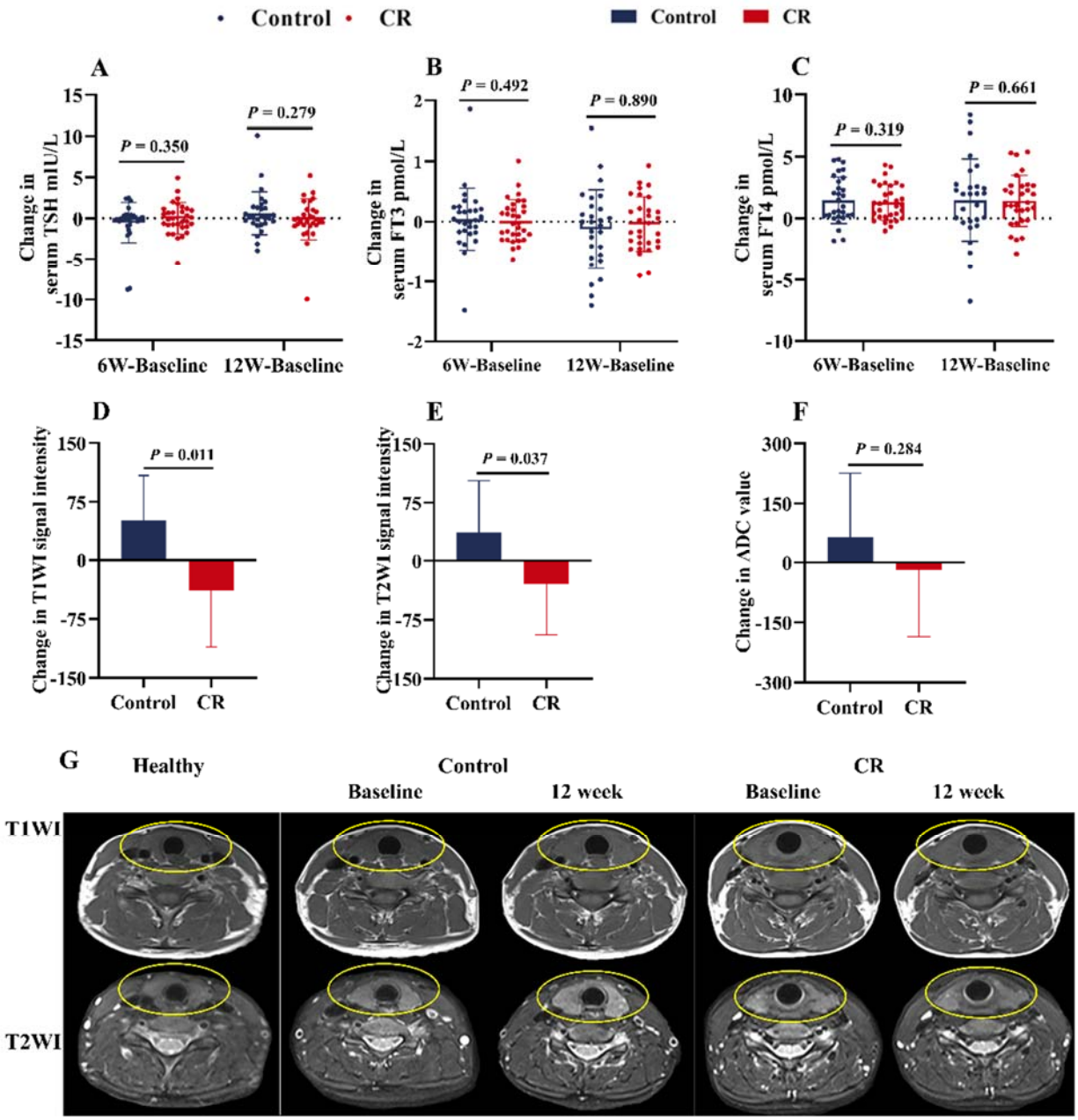

Figure 3. Effects of CR diet on function and morphological assessment of thyroid

A-C: Changes of TSH, FT3 and FT4 levels from baseline to the middle and the end of intervention in the CR group and the control group. D-F: Mean changes of T1WI, T2WI signal intensities and ADC value from baseline to the end of intervention in the CR group and the control group. G: T1WI and T2WI images of healthy subjects and participants at baseline and the end of intervention in both groups. Thyroid gland was highlighted with a yellow ellipse. Between-group significance was analyzed with two-tailed unpaired t test. Abbreviations: CR, calorie restricted; TSH, thyroid-stimulating hormone; FT3, free triiodothyronine, FT4, free thyroxine; T1WI, T1 weighted image; T2WI, T2 weighted image, ADC, apparent diffusion coefficient.

3.4 Effects of CR diet on Hashimoto's thyroiditis symptom

The scores of severity of multiple symptoms were significantly decreased after CR diet for 12 weeks, especially the symptoms related to digestive system, such as abdominal distension, constipation, diarrhea and hiccup. In addition, symptoms of chilliness, gain weight, facial edema, dizziness, forgetfulness, anxiety,

depressed, numbness and infertility were significantly improved by CR diet (Table S5). Furthermore, correlation analysis showed that the improvements in symptoms of abdominal distension, constipation, hiccup, chilliness, gain weight, facial edema, forgetfulness, anxiety, depressed, numbness and infertility were positively correlated with the reduction of TPO-Ab (Table 2).

3.5 Effects of CR diet on relative count of T lymphocyte subpopulations in blood

To evaluate the effects of CR diet on immune function, relative counts of T lymphocyte subpopulations in peripheral blood were determined. The changes of percentages of $CD3^+$ T lymphocyte/total lymphocyte (3.80%; 95% CI: 1.64 to 5.97) and $CD4^+$ T lymphocyte/total lymphocyte (3.08%; 95% CI: 0.29 to 5.86) from baseline to 12-week were significantly increased in the CR group, compared with that in the control group (Table 2). There was no difference in percentages of $CD8^+$ T lymphocyte/total lymphocyte or ratio of $CD4^+/CD8^+$ (Table 2).

Table 2 The relationship between TPO-Ab, TG-Ab changes and symptom scores changes in 12−week

Change of scores	Changes of TPO-Ab			Changes of TG-Ab		
	r value	95% CI	*P* value	r value	95% CI	*P* value
Digestive system	0.271	-0.002 to 0.506	0.045	0.181	-0.092 to 0.428	0.179
Anorexia	0.037	-0.235 to 0.305	0.784	0.036	-0.232 to 0.298	0.791
Abdominal distension	0.364	0.101 to 0.579	0.006	0.098	-0.174 to 0.357	0.468
Constipation	0.307	0.037 to 0.535	0.023	0.231	-0.039 to 0.470	0.084
Diarrhea	0.190	-0.087 to 0.440	0.164	0.222	-0.049 to 0.463	0.097
Gastrointestinal cramps	0.209	-0.067 to 0.456	0.125	-0.019	-0.286 to 0.250	0.886
Acid reflux	0.176	-0.102 to 0.428	0.199	0.098	-0.175 to 0.356	0.470
Burning sensation	0.071	-0.205 to 0.338	0.605	-0.010	-0.277 to 0.259	0.942
Hiccup	0.312	0.043 to 0.539	0.020	0.253	-0.016 to 0.488	0.058
Respiratory system	-0.030	-0.301 to 0.245	0.826	0.341	0.080 to 0.558	0.009
Cough	-0.061	-0.329 to 0.215	0.657	0.169	-0.104 to 0.418	0.210
Asthma	0.073	-0.204 to 0.339	0.595	0.442	0.197 to 0.635	0.001
Circulatory system	0.390	0.131 to 0.599	0.003	0.086	-0.187 to 0.346	0.526
Bradycardia	0.256	-0.018 to 0.494	0.059	0.183	-0.090 to 0.429	0.174
Palpitations	0.244	-0.031 to 0.485	0.073	0.090	-0.182 to 0.350	0.505
Chest tightness	0.227	-0.049 to 0.471	0.096	0.042	-0.229 to 0.306	0.759
Hypotension	0.354	0.090 to 0.572	0.008	0.117	-0.156 to 0.373	0.386
Endocrine system	0.412	0.154 to 0.617	0.002	0.185	-0.090 to 0.433	0.173
Chilliness	0.419	0.165 to 0.621	0.001	0.060	-0.211 to 0.323	0.658
Gain weight	0.286	0.011 to 0.520	0.036	0.061	-0.213 to 0.326	0.657
Facial edema	0.332	0.062 to 0.556	0.014	0.266	-0.004 to 0.501	0.047
Weight loss	0.063	-0.216 to 0.333	0.649	0.261	-0.011 to 0.496	0.052
Parched	0.200	-0.079 to 0.451	0.147	0.198	-0.077 to 0.444	0.144
Sweating	0.179	-0.101 to 0.433	0.195	-0.068	-0.332 to 0.206	0.618
Neuroscience system	0.260	-0.014 to 0.497	0.055	0.154	-0.118 to 0.405	0.252
Headache	0.132	-0.146 to 0.390	0.338	-0.086	-0.346 to 0.186	0.524
Dizziness	0.248	-0.027 to 0.487	0.068	0.067	-0.204 to 0.329	0.619
Forgetfulness	0.352	0.088 to 0.570	0.008	0.145	-0.128 to 0.397	0.283
Anxiety	0.348	0.083 to 0.567	0.009	0.089	-0.183 to 0.349	0.511
Depressed	0.298	0.028 to 0.528	0.027	0.057	-0.214 to 0.320	0.673

Fatigue	0.081	-0.196 to 0.346	0.555	0.053	-0.218 to 0.316	0.695
Insomnia	-0.123	-0.383 to 0.155	0.373	-0.017	-0.283 to 0.252	0.901
Multiple dreams	-0.131	-0.390 to 0.147	0.340	0.112	-0.161 to 0.369	0.408
Drowsiness	0.147	-0.131 to 0.404	0.284	0.083	-0.189 to 0.344	0.538
Irritability	-0.029	-0.299 to 0.246	0.835	0.126	-0.147 to 0.381	0.350
Indifferent	0.114	-0.164 to 0.375	0.409	0.015	-0.254 to 0.282	0.913
Movement system	0.384	0.125 to 0.595	0.004	0.099	-0.173 to 0.358	0.462
Joint pain	0.111	-0.166 to 0.373	0.418	0.024	-0.245 to 0.290	0.858
Muscle pain	0.297	0.027 to 0.527	0.028	-0.034	-0.299 to 0.236	0.801
Swelling	0.380	0.120 to 0.592	0.004	-0.137	-0.391 to 0.136	0.309
Numbness	0.361	0.098 to 0.577	0.007	0.091	-0.181 to 0.351	0.499
Reproductive system	0.276	0.001 to 0.512	0.044	0.193	-0.081 to 0.441	0.153
Menorrhagia	0.293	0.022 to 0.524	0.030	0.221	-0.049 to 0.462	0.098
Oligomenorrhea	0.102	-0.175 to 0.365	0.457	0.198	-0.074 to 0.443	0.139
Infertility	0.412	0.157 to 0.616	0.002	-0.057	-0.320 to 0.214	0.672
Miscarriage	0.179	-0.101 to 0.433	0.195	-0.094	-0.356 to 0.180	0.489
Mucocutaneous	0.120	-0.160 to 0.383	0.387	0.095	-0.179 to 0.357	0.484
Dry skin	0.002	-0.271 to 0.275	0.989	-0.037	-0.302 to 0.233	0.783
Pruritus	0.033	-0.242 to 0.303	0.812	0.111	-0.161 to 0.368	0.409
Acne	0.070	-0.207 to 0.336	0.613	-0.015	-0.281 to 0.254	0.914
Dermatitis	0.026	-0.248 to 0.297	0.850	0.149	-0.124 to 0.401	0.269
Subcutaneous bleeding	0.250	-0.025 to 0.489	0.066	0.207	-0.065 to 0.450	0.123
Mouth ulcers	0.152	-0.126 to 0.408	0.268	-0.092	-0.351 to 0.181	0.497
Photosensitivity	0.135	-0.143 to 0.393	0.327	0.175	-0.097 to 0.423	0.193
Hair loss	0.060	-0.217 to 0.327	0.665	0.002	-0.266 to 0.269	0.990
Eyebrows fall out	0.079	-0.201 to 0.346	0.572	0.095	-0.180 to 0.356	0.485
Fragile nails	-0.042	-0.312 to 0.233	0.758	-0.156	-0.407 to 0.117	0.246

Data are derived from Spearman correlation coefficient. The significant level was $P < 0.05$.
Abbreviations: TPO-Ab, antithyroid peroxidase; TG-Ab, antithyroid thyroglobulin.

3.6 Effects of CR diet on inflammatory biomarkers

Changes of IFN-γ (-0.81 pg/mL; 95% CI: -1.07 to -0.54), IL-4 (-0.30 pg/mL; 95% CI: -0.58 to -0.03), IL-6 (-0.28 pg/mL; 95% CI: -0.49 to -0.06) and IL-17A (-0.31 pg/mL; 95% CI: -0.58 to -0.05) from baseline to 6 weeks were all significantly reduced in the CR arm compared with the control arm (Table 3). In addition, change of IL-10 in the CR group was significantly higher than that in the control arm (0.36 pg/mL; 95% CI: 0.16 to 0.56). While, at 12 weeks, only the changes of IL-10 (-0.69 pg/mL; 95% CI: -1.31 to -0.07) showed a significant between-group difference (Table 3). There was no between-group difference in change of TNF-α from baseline to 6 weeks or to 12 weeks (Table 3). Correlation analysis showed that the reductions of IL-10 and IL-17A were both positively correlated with the reduction of TPO-Ab level (Table S6).

3.7 Effects of CR diet on serum lipid levels

TC changes in the CR group were -0.44 mmol/L (95% CI: -0.68 to -0.19) at 6-week, and were -0.36 mmol/L (95% CI: -0.65 to -0.07) at 12-week, both with a significant between-group difference (Table 3). LDL-C was significantly reduced in the CR group compared with the control group (-0.25 mmol/L; 95% CI,

-0.49 to -0.01) at 12 weeks (Table 3). No significant changes of TG or HDL-C were observed in the study groups (Table 3).

Table 3 Changes in thyroid function, lipids, inflammatory factors and immune biomarkers during the intervention period

Variables	Calorie Restriction Group (n = 33)	Control Group (n = 33)	Difference between Groups (95% CI)
	Change from baseline (95% CI)		
TC, mmol/L			
6 weeks	-0.38 (-0.97 to 0.21)	0.05 (-0.27 to 0.37)	-0.44 (-0.68 to -0.19)
12 weeks	-0.37 (-0.94 to 0.2)	-0.01 (-0.53 to 0.51)	-0.36 (-0.65 to -0.07)
TG, mmol/L			
6 weeks	0.15 (-0.15 to 0.45)	0.11 (-0.23 to 0.45)	0.04 (-0.12 to 0.21)
12 weeks	0.12 (-0.26 to 0.50)	0.11 (-0.28 to 0.50)	0.01 (-0.19 to 0.21)
LDL-C, mmol/L			
6 weeks	-0.19 (-0.86 to 0.48)	-0.08 (-0.33 to 0.17)	-0.12 (-0.38 to 0.15)
12 weeks	-0.39 (-0.88 to 0.10)	-0.13 (-0.54 to 0.28)	-0.25 (-0.49 to -0.01)
HDL-C, mmol/L			
6 weeks	-0.08 (-0.32 to 0.16)	-0.02 (-0.20 to 0.16)	-0.07 (-0.17 to 0.04)
12 weeks	-0.10 (-0.40 to 0.20)	0.05 (-0.33 to 0.43)	-0.15 (-0.33 to 0.03)
TNF-α, pg/mL			
6 weeks	-0.23 (-0.66 to 0.2)	-0.11 (-0.55 to 0.33)	-0.12 (-0.35 to 0.10)
12 weeks	0.33 (0.25 to 0.91)	0.85 (-0.54 to 2.24)	-0.52 (-1.08 to 0.03)
IFN-γ, pg/mL			
6 weeks	-0.64 (-1.11 to -0.17)	0.16 (-0.4 to 0.72)	-0.81 (-1.07 to -0.54)
12 weeks	0.44 (-3.01 to 3.89)	0.04 (-2.01 to 2.09)	0.40 (-1.12 to 1.91)
IL-4, pg/mL			
6 weeks	-0.24 (-0.57 to 0.09)	0.07 (-0.63 to 0.77)	-0.30 (-0.58 to -0.03)
12 weeks	0.85 (0.06 to 1.64)	1.06 (-1.16 to 2.28)	-0.21 (-0.74 to 0.33)
IL-6, pg/mL			
6 weeks	-0.62 (-1.06 to -0.18)	-0.35 (-0.74 to 0.04)	-0.28 (-0.49 to -0.06)
12 weeks	1.18 (0.26 to 2.1)	1.38 (-0.2 to 2.96)	-0.20 (-0.88 to 0.48)
IL-10, pg/mL			
6 weeks	0.01 (-0.39 to 0.41)	-0.34 (-0.73 to 0.05)	0.36 (0.16 to 0.56)
12 weeks	0.52 (-0.18 to 1.22)	1.21 (-0.32 to 2.74)	-0.69 (-1.31 to -0.07)
IL-17A, pg/mL			
6 weeks	-0.52 (-1.11 to 0.07)	-0.20 (-0.61 to 0.21)	-0.31 (-0.58 to -0.05)
12 weeks	0.13 (-1.15 to 1.41)	0.87 (-0.84 to 2.58)	-0.74 (-1.54 to 0.05)
$CD3^+$/total lym, %			
6 weeks	2.58 (-1.29 to 6.45)	1.52 (-0.83 to 3.87)	1.06 (-0.61 to 2.73)
12 weeks	2.59 (-1.31 to 6.49)	-1.21 (-5.47 to 3.05)	3.80 (1.64 to 5.97)
$CD4^+$/total lym, %			
6 weeks	3.19 (-1.37 to 7.75)	2.21 (-0.9 to 5.32)	0.99 (-1.05 to 3.02)
12 weeks	2.90 (-2.29 to 8.09)	-0.18 (-5.46 to 5.1)	3.08 (0.29 to 5.86)
$CD8^+$/total lym, %			
6 weeks	-0.16 (-2.49 to 2.17)	-0.31 (-2.85 to 2.23)	0.15 (-1.11 to 1.41)
12 weeks	0.24 (-3.33 to 3.81)	0.21 (-2.65 to 3.07)	0.03 (-1.69 to 1.75)
$CD4^+/CD8^+$, ratio			
6 weeks	0.15 (-0.12 to 0.42)	0.12 (-0.15 to 0.39)	-0.03 (-0.11 to 0.17)
12 weeks	0.11 (-0.23 to 0.45)	0.01 (-0.31 to 0.31)	0.11 (-0.06 to 0.28)

Between-group differences in the change from baseline to the end of the study were tested with unpaired *t* tests. Mean differences are expressed with their 2-sided 95% CIs. Abbreviations: TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TNF-α, tumor necrosis factor α; IFN-γ, interferon γ; IL, interleukin.

4. Discussion

To the best of our knowledge, the present RCT study is the first trial to investigate the effect of CR on HT patients. We found that 12 weeks of CR diet significantly decreased the primary efficacy end point of TPO-Ab and TG-Ab in HT patients compared with control diet. Moreover, CR significantly improved non-hypothyroid symptoms and immune dysfunction, as well as decreased levels of pro-inflammatory biomarkers and serum lipids.

HT is a T-cell-mediated disease of unknown cause, characterized by abnormal elevation in TPO-Ab and/or TG-Ab. Clinically, TPO-Ab is considered with higher sensitivity and specificity than TG-Ab in the diagnosis of autoimmune thyroid disease [31-32]. HT patients always report diverse symptoms despite adequate thyroid hormone replacement (such as levothyroxine), thus these symptoms are thought to be related to autoimmune disease rather than to hypothyroidism [33-34]. Higher antibodies levels have been associated with an increased symptom load and a decreased quality of life in an euthyroid HT patient collective [34]. A possible mechanism exists that activated anti-TPO/TG antibody-producing lymphocytes may leave thyroid gland and invade other distant tissue, contributing to non-hypothyroid symptoms [35-36]. Hence, it's the key to lower the TPO-Ab and TG-Ab levels for HT recovery. In our results, CR diet significantly decreased serum TPO-Ab and TG-Ab levels, accompanied by an improvement in the associated symptoms, particularly in abdominal distension, constipation, diarrhea, hiccup, facial edema, forgetfulness and anxiety. Although between-group differences in antibody reductions were significant, within-group comparisons in the CR group did not consistently reach significance, likely due to the relatively small sample size and high inter-individual variability. Even so, a higher proportion of participants in the CR group showed meaningful antibody decreases compared with controls. Given the modest effect sizes and multiple comparisons, these findings should be regarded as exploratory, and longer-term studies are required to establish their clinical relevance. Furthermore, improvement in symptoms was positively correlated with the decrease in antibodies, suggesting that decreasing antibodies might effectively improve multiple symptoms.

Furthermore, improvement in symptoms was positively correlated with the decrease in antibodies, suggesting that decreasing antibodies might effectively improve multiple symptoms. Given that the CR diet is easily reproducible and has the potential to be applied in clinical, these results are quite encouraging and promising for HT patients.

The precise mechanisms by which CR decreased TPO-Ab and TG-Ab levels are not clear, but likely involve immunological regulation [37-39]. $CD3^+$ is a marker of all T-lymphocyte subpopulations, directly reflecting the overall immunity level of the organism, and its reduction indicates a decrease in the overall immunity of the organism [40-42]. $CD4^+$ is a marker of T helper/inducer cells and an important indicator of immune response, and its decrease is mostly seen in viral infectious diseases. Our results showed that CR diet significantly increased the contents of $CD3^+$ and $CD4^+$ T lymphocyte compared with control diet, indicating the immunity improvement effect of CR diet on HT patients. This conclusion was consistent with that from a recent RCT, which investigated the thymic function changes after 2 years of continuous CR among

middle-aged healthy participants by magnetic resonance imaging (MRI) and blood biomarkers test, showing that CR effectively increased thymic volume, affect $CD4^+$ cell homeostasis, and improved immune function of the body [21].

CR has been shown to lower inflammation, partly by reducing the expression of T-lymphocyte-derived pro-inflammatory cytokines, such as IL-6, IL-4 and IFN-γ [24-26], [43]. Of interest, higher levels of these cytokines have been observed in HT patients than healthy subjects [37]. Moreover, reduced serum TPO-Ab was correlated with decreased levels of IFN-γ, TNF-α, IL-1β and IL-2, as well as C-reactive protein [44]. In the present study, in addition to the reduction of these cytokines, we firstly reported that CR diet of 6 weeks induced a significant decrease in IL-17A, a kind of pro-inflammatory cytokines secreted by Thl7 [45]. It has been reported that the expression of IL-17A in thyroid tissue and peripheral blood of HT patients is higher than that of normal individuals [46]. Thus, it is reasonable to presume that decreasing the aforementioned cytokines may be potential targets for HT treatment.

However, our results showed a significant reduction in inflammatory biomarkers at 6 weeks, which was not sustained at 12 weeks. These may be explained by several factors. First, the COVID-19 outbreak occurred in our region during the 6-12 weeks period, and infection-related stress or subclinical immune responses could have influenced systemic inflammation at follow-up. Second, physiological adaptation to prolonged calorie restriction and declining dietary adherence may also have attenuated the observed effects. These findings emphasize the need for caution when interpreting time-dependent changes in inflammatory outcomes under external stressors.

In recent years, CR has become one of the most widely researched interventions showing promise in improving multiple chronic metabolic diseases, such as cardiovascular, type 2 diabetes, dyslipidemia and cancer [13] [24] [26]. Notably, several studies have identified its clear benefits on metabolic risk factors, in terms of reducing body weight, cholesterol level, blood pressure and post-prandial glycemia [47-48]. For example, in a RCT enrolled 139 obesity patients, CR diet significantly decreased body weight, serum TG, TC and LDL-C [17]. Consistent with previous studies on aforementioned population, our results for the first time showed that CR effectively reduced body weight, serum TC and LDL-C in HT patients, even though these patients were often accompanied by abnormal lipid metabolism and reported they are hard to lose weight [49-51].

Strengths of the present study included its randomized design, perfect follow-up with few dropouts and all-round testing indicators. Furthermore, we used MRI technology to provide a more accurate assessment of thyroid gland, which allowed us to observe subtler changes before and after the intervention. Increases in T1WI and T2WI signal intensity have been associated with destruction of thyroid gland [52-53], and our results showed that CR can bring the thyroid parameters of HT patients towards those of healthy individuals.

Nevertheless, our study has several limitations. First, the trial was performed at a single research site, resulting in limited sampling of geographical and ethnic backgrounds. Second, the duration of the intervention was not long enough (3 months). The brevity of the intervention period could potentially account for the observed stability in thyroid hormone levels, which showed an expected trend of change but failed to exhibit

significant variation. Third, our study was not fully double-blinded due to the inherent difficulties in this type of study. Participants were inevitably aware of their group assignment, which may have introduced reporting bias in subjective outcomes such as quality-of-life assessments. While objective biochemical outcomes are less likely to be influenced, the interpretation of self-reported data should be made with caution. To minimize potential biases, we adopted several measures: (1) the meal plans provided to participants specified only the weight of each ingredient without revealing caloric content, thereby preventing participants from easily discerning their group allocation; (2) participants in different groups were managed in two separate WeChat groups to avoid cross-communication; and (3) the researchers responsible for estimating caloric intake from food photos and those analyzing the serum biomarkers and questionnaires were independent teams, both blinded to group assignments.

5. Conclusion

In conclusion, the present RCT found that CR significantly reduced serum TPO-Ab and TG-Ab levels in HT patients, lightened the non-hypothyroid symptom burden, simultaneously showed a positive impact on immune dysfunction, inflammatory biomarkers and lipids. This study provides important evidence of CR diet for decreasing thyroid antibodies in HT patients, although more replications with larger sample size and longer intervention time are still warranted. Importantly, while calorie restriction can be feasible and beneficial, attention to micronutrient adequacy remains essential for clinical translation.

Conflict of interest

The authors have no conflict of interest to declare.

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Ethic approval and consent to participate

We declare that the ethical background to this study was approved by the Ethics Committee of Medical ethics at Zhejiang Chinese Medical University (No. 20220607-2). Written informed consent was provided by each participant.

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