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# The Effect of Hypothyroidism on Color Contrast Sensitivity: A Prospective Study

Mehtap Cakir[b] Banu Turgut Ozturk[a] Elif Turan[b] Gulsum Gonulalan[b]
Ilker Polat[c] Kemal Gunduz[d]

[a]Department of Ophthalmology, School of Medicine, Selcuk University, [b]Division of Endocrinology and Metabolism, and Departments of [c]Internal Medicine and [d]Ophthalmology, Meram School of Medicine, Konya Necmettin Erbakan University, Konya, Turkey

**Key Words**
Thyroid · Hypothyroidism · Opsin · Cone · Color vision

**Abstract**
***Background:*** Thyroid hormone has been shown to control retinal cone opsin expression, the protein of color vision, in adult rodents. ***Objectives:*** The aim of this study was to evaluate the effect of hypothyroidism on color contrast sensitivity in adult overt hypothyroid patients. ***Methods:*** Thirty-eight overt hypothyroid (31 females, 7 males) subjects and 20 euthyroid (16 females, 4 males) controls were studied prospectively. Color vision examination was performed by Chromatest, a software program analyzing the tritan (blue-yellow) color contrast threshold (tritan CCT) and protan (red-green) color contrast threshold (protan CCT). Color contrast sensitivity analyses of hypothyroid subjects were performed on admission and after L-thyroxine treatment when biochemical euthyroidism was achieved. ***Results:*** After a median period of 90 (90–210) days, 24 (19 females, 5 males) patients were euthyroid and eligible for a second color vision examination. Baseline tritan CCT and protan CCT values were significantly higher in the hypothyroid group compared to euthyroid controls, which clinically translates into impaired color contrast sensitivity ($p < 0.001$ and $p < 0.001$, respectively). There was a significant decrease in tritan CCT ($p = 0.002$) and protan CCT ($p < 0.001$) values in the hypothyroid group after euthyroidism was achieved, which denotes improvement in color contrast sensitivity. ***Conclusions:*** It is a novel finding of the current study that color contrast sensitivity is impaired in hypothyroidism and significantly improves after euthyroidism is achieved.



## Introduction

The retina is the ocular tissue layer that converts light into electrical signals. Cone cells in human retina mediate daylight vision and are critical for visual acuity and color discrimination. There are three cone types termed L, M and S, which are mainly distinguished by their sensitivity to the portion of the visible spectrum. L, M and S cones are most sensitive to long (red color), middle (green color) and short (blue color) wavelengths, respectively. The difference between cones depends on the expression of the visual pigments expressed in cone photoreceptors which are made up of a large protein component called opsin [1]. Although most mammals including mice dif-

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E-Mail karger@karger.com
www.karger.com/etj

Mehtap Cakir, MD
Konya Necmettin Erbakan University
Division of Endocrinology and Metabolism
TR–42080 Konya (Turkey)
E-Mail cakirmehtap@yahoo.com

**Table 1.** Baseline characteristics and Chromatest results of the hypothyroid and euthyroid control groups

| Parameter | Baseline hypothyroid group (n = 38) | Prospective hypothyroid group (n = 24) before treatment | Prospective hypothyroid group (n = 24) after treatment | Euthyroid control group (n = 20) | p value |
|---|---|---|---|---|---|
| Age, years[a] | 40.53±13.33 | 38.92±13.41 | 38.92±13.41 | 35.10±7.71 | 0.06[b], 0.25[c] |
| Females/males | 31/7 | 19/5 | 19/5 | 16/4 | 1.0[b, c] |
| Free T3[a] (0.02–0.05 pmol/l) | 0.04 (0.004–0.05) | 0.04 (0.02–0.05) | 0.05 (0.04–0.06) | 0.05 (0.04–0.06) | 0.002[b], <0.001[c], 0.29[d] |
| Free T4[a] (7.9–14.4 pmol/l) | 6.17 (0.90–7.72) | 6.30 (0.90–7.72) | 11.32 (7.97–16.08) | 10.42 (8.11–12.36) | <0.001[b, c], 0.11[d] |
| TSH[a] (0.34–5.6 mU/l) | 25.63 (6.70–100) | 26.24 (6.70–100) | 1.38 (0.53–5.53) | 1.39 (0.61–3.59) | <0.001[b, c], 0.24[d] |
| Anti-TPO positivity, % | 81.60 | 87.50 | | | |
| Anti-Tg positivity, % | 68.40 | 70.80 | | | |
| Tritan CCT (blue-yellow), %[a] | 5.45 (2.80–23.40) | 5.60 (2.80–10.80) | 4.80 (2.60–10.50) | 4.05 (2.60–6.30) | <0.001[b–d], 0.002[e] |
| Protan CCT (red-green), %[a] | 2.00 (1.00–4.90) | 2.00 (1.00–3.90) | 1.70 (1.00–3.40) | 1.60 (0.90–2.70) | <0.001[b, c, e], 0.07[d] |

Anti-TPO = Anti-thyroperoxidase; anti-Tg = anti-thyroglobulin. [a] Median (range). [b] Baseline hypothyroid group vs. control group. [c] Prospective hypothyroid group before treatment vs. control group. [d] Prospective hypothyroid group after treatment vs. control group. [e] Prospective hypothyroid group before treatment vs. prospective hypothyroid group after treatment.

ferentially express M and S opsins for response to medium-long and short wavelengths and thus are dichromatic, primates including humans, have evolved trichromatic color vision [1, 2].

It is well established that thyroid hormone (TH) is critical for normal brain development including development of structures necessary for visual processing [3]. In mouse retinal development, TH through its receptor TRβ2 is an important regulator of cone spectral identity by repressing S opsin and activating M opsin [4–9]. In the human fetus, TRβ2 is detected in cones at gestational week 12 and in human fetal retinal cell cultures, TH promotes cone photoreceptor differentiation [10, 11]. In human Weri retinoblastoma cells which express TRβ2, T3 can induce L/M opsin mRNA [12, 13]. To our knowledge, there are no systematic studies that address the consequences of hypothyroidism on human color contrast sensitivity in adults.

Color vision, which is described as the absorption of light by three different spectral classes of cones may be evaluated by several methods. In clinical practice, color vision is usually determined by Ishihara plates, which is not a quantitative method. The most objective test to evaluate the function of cones is electroretinogram which is a long and invasive test and warrants foil electrodes to be placed on the eyes [14]. Alternatively, color contrast sensitivity, which is another parameter of color vision, may accurately be measured by Chromatest [15]. Accordingly, the aim of the current study was to determine the effect of hypothyroidism on color contrast sensitivity by Chromatest in adult overt hypothyroid patients before and after euthyroidism is achieved and compare their color contrast sensitivity examination results with a euthyroid control group.

## Materials and Methods

*Participants*

Thirty-eight (31 females, 7 males) overt hypothyroid drug-naïve patients (baseline hypothyroid group) who were admitted to the Endocrinology and Metabolism outpatient clinic and 20 (16 females, 4 males) age- and gender-matched euthyroid control subjects (euthyroid control group) from the hospital staff were recruited and studied prospectively (table 1). The study was approved by The Ethics Committee of School of Medicine, and all participants provided written informed consent.

In the hypothyroid group, the etiologies of hypothyroidism included Hashimoto's thyroiditis (HT) and postoperative hypothyroidism due to surgery for nodular goiter. The diagnosis of HT was established on biochemical grounds (by measuring serum free T3, free T4 and TSH), positive anti-thyroperoxidase and/or anti-thyroglobulin levels as well as sonographic findings [16]. Patients with overt hypothyroidism defined as low free T3 (0.02–0.05 pmol/l) and/or low free T4 (7.9–14.4 pmol/l) combined with high TSH (0.34–5.6 mU/l) levels were included in the study.

*Procedures*

Ophthalmological examination including visual acuity, intraocular pressure measurement, biomicroscopy and fundus examination were performed in all patients, and those with any ocular pathology were excluded from the study as it may affect visual acuity and color vision [17]. To evaluate the status of color contrast sensitivity, each patient underwent a color contrast sensitivity testing. Chromatest is a software program analyzing the age-corrected tritan (blue-yellow) color contrast threshold (tritan CCT) and protan (red-green) color contrast threshold (protan CCT). In this program, the letters are displayed on a background of equiluminance. A brief explanation of what the patient is expected to see and their expected response was made prior to the test. The right eye was tested first followed by the left.

For the Chromatest, the subject is seated at a fixed distance from the computer, and an alphabetical letter is displayed on the screen. The test is self-calibrating and automatic, so the operator (ophthalmologist) has no influence on the contrast of the test letter given. The operator waits for the answer of the patient for the current letter on the screen and then clicks on the correct or incorrect button on the operator screen according to the subject's response. If the response is correct, on the next presentation the color contrast difference between the letter and background is halved. If the response is incorrect, the color contrast is doubled. By using this method, the computer determines thresholds that lead to finite steps which reach a plateau at the color contrast sensitivity threshold. Cognitive functions may be impaired in hypothyroidism, which may lead to slowed reaction times. To rule out this bias, when the subjects were being tested with Chromatest, enough period of time to answer was given, and after they have clearly mentioned what they see, the contrast level of the letter on the screen has been changed. Thus, the response period of the patient does not have an impact on the final threshold or sensitivity of the test.

*Testing Protocol*

After the first ophthalmological examination, L-thyroxine treatment was started in hypothyroid patients. Six weeks later, serum free T4 and TSH levels were checked and titration of L-thyroxine dose was performed if necessary (data not shown). Another 6 weeks later (minimum 3 months after admission), thyroid function tests including free T3, free T4 and TSH were checked and if the results were within the euthyroid range, the patient's ophthalmological examination was re-performed. In patients who needed further dose adjustments, ophthalmological examinations were postponed until they became biochemically euthyroid. From the baseline hypothyroid group (n = 38), 14 patients were lost to follow-up and a second color vision analysis was possible in a subgroup of 24 (19 females, 5 males) patients after a median period of 90 (90–210) days. This patient subgroup was named as treated hypothyroid group for ease of understanding, and their characteristics and Chromatest results are given under two separate headings: treated hypothyroid group before treatment and treated hypothyroid group after treatment (table 1).

*Statistics*

Data were analyzed using the Statistical Package for the Social Sciences (SPSS version 13.0) for Windows. Differences between baseline versus treated hypothyroid groups and hypothyroid groups versus controls were analyzed using Fisher's exact test, Student's t test or Mann-Whitney U test depending on normality test results. The protan CCT and tritan CCT values before and after treatment were compared with controls and each other with the Mann-Whitney U test after validation of normality with the Kolmogorov-Smirnov test. Data were also analyzed further within the group using the Wilcoxon signed-rank test. The correlations between study parameters were analyzed by Spearman's correlation test. All tests were performed at an error level of 5%.

## Results

Table 1 shows the baseline characteristics of the hypothyroid and control groups. There was statistically no significant difference in age and sex distribution between both the baseline and treated hypothyroid groups and the euthyroid control group.

The ophthalmological examination of all patients which was performed before and after the treatment revealed normal findings including a negative relative afferent pupillary defect, which excludes a possible optic neuropathy-related color vision abnormality. Table 1 shows Chromatest results of the hypothyroid and control groups. When interpreting tritan CCT and protan CCT values, higher values clinically translate into impaired color contrast sensitivity. Tritan CCT and protan CCT values of the baseline hypothyroid group and the treated hypothyroid group before treatment were significantly higher compared to the euthyroid control group ($p < 0.001$ and $p < 0.001$ for both groups, respectively). After hypothyroid patients were given treatment and euthyroidism was achieved, there was a significant decrease in both tritan CCT and protan CCT values ($p = 0.002$ and $p < 0.001$, respectively), which corresponds to improved blue-yellow and red-green color contrast sensitivity (table 1). There was no correlation between the difference of pre- and posttreatment TSH levels and the difference of pre- and posttreatment protan CCT ($r = 0.13$, $p = 0.36$) and tritan CCT ($r = 0.25$, $p = 0.08$) levels. Pre- and posttreatment protan CCT and tritan CCT changes were also not correlated ($r = 0.27$, $p = 0.06$). When these euthyroid tritan CCT and protan CCT values were compared with the control group's values (treated hypothyroid group after treatment vs. euthyroid control group), the significant difference between hypothyroid and control groups' tritan CCT values remained ($p < 0.001$). In other words, the significant impairment in red-green color contrast sensitivity (protan CCT) had resolved but impaired blue-yellow color contrast sensitivity (tritan CCT), although diminished, still persisted.

## Discussion

To our knowledge, this study is the first to show overt hypothyroidism may impair color contrast sensitivity and after 3 months of treatment for hypothyroidism impaired color vision may improve to some extent.

In the literature, there are 2 adult animal studies in which the role of TH in the maintenance of *mature* cone photoreceptor pattern by late-onset hypothyroidism were evaluated [6, 18]. In the first study by Applebury et al. [6], after subjecting adult mice to anti-thyroid drug treatment for 2 weeks, no effect on cone opsin expression was found. In the study by Glaschke et al. [18], methimazole-induced suppression of serum TH in adult mice and rats yielded no changes in cone numbers but reversibly altered cone patterns by activating the expression of S opsin and suppressing the expression of M-cone opsin 5–7 weeks after serum TH concentrations had decreased to hypothyroid levels. The results of this study challenge the idea that, after maturation TH has no effect on cone opsin expression, the spectral identity and once specified retinal distribution of S and M cones were rigidly preserved [18]. The discrepancy between these 2 studies' results has been attributed to the relatively shorter anti-thyroid drug treatment period in the study by Applebury and colleagues. It has been reported that central neural tissue maintains physiological levels of T3 for some time under conditions of peripheral hypothyroidism, and a longer period is needed for central neural tissue level hypothyroidism [19]. These 2 studies, to some extent, may serve as appropriate preclinical models for our study. In an attempt to adapt the preclinical study findings by Glaschke and colleagues, one of the expected results in our study would be impairment in green color vision due to decreased M opsin expression (increased protan CCT values) in hypothyroidism followed by an improvement after restoration of euthyroidism [18]. Thus, regarding protan CCT measurements, the expected changes were noted in the current study. However, either improvement or at least insignificant changes were expected to occur in blue color vision due to activated S opsin expression (increased tritan CCT values) in the hypothyroid state which is in conflict with our findings. With current literature data, the only possible explanation for this finding may be differences in opsin expression patterns between adult dichromatic mice and human retina.

We are not aware of any clinical studies in the literature evaluating the effect of adult hypothyroidism on color vision. On the other hand, in a study on preterm infants, Simic et al. [20] reported slow blue-yellow and red-green color vision enhancement. Also, albeit rare, male patients are color blind in TH resistance syndrome, which represents a model for fetal hypothyroidism. In a case report, reduced L- and M- and increased S-cone functions in an infant with TH resistance due to mutations in the THRβ2 gene were demonstrated [21]. However, neither fetal hypothyroidism nor preterm infant studies may serve as appropriate models for adult hypothyroidism. The reason is TH effects on the developing fetus change depending on the time frame of TH deficiency. In this regard, transient hypothyroxinemia of prematurity has been linked to suboptimal neurodevelopment [20]. Similarly, cone differentiation and opsin expression are already effected in the fetal period in TH resistance syndrome.

Humans have a duplicated array of L and M locus opsin genes for long- and medium-wave responses [22]. Results from molecular genetic studies have revealed that the L and M opsin genes were adjacent to one another with no intervening genes and localized to the X-chromosome, and the gene for the S opsin to an autosome, chromosome 7 [23]. Nathans et al. [24] have discovered a DNA element upstream of the L opsin gene that is essential for the transcription of the X-chromosome opsin genes. The DNA element was given the name locus control region (LCR). LCR is a highly conserved enhancer that mediates cell type-specific expression of the X-chromosome opsin genes [25]. LCR is present in all other mammalian species examined, the vast majority of which have a single X-chromosome opsin gene. In this regard, hypothetically hypothyroidism may retard the expression of both L and M opsins by affecting the common LCR. When our study results are considered in this context, decrements of both tritan CCT (blue-yellow) and protan CCT (red-green) levels in the hypothyroid group may imply a negative influential and duration-dependent effect of TH deficiency on all three opsins, namely L, M and S.

There are a few limitations of the current study. The first one is the design of the study. To eradicate the possibility of a learning effect, it would definitely be more clarifying if the control subjects could also be tested for a second time. The findings of the hypothyroid group are thought to be genuinely due to treatment of hypothyroidism not to the learning effect as protan CCT and tritan CCT values did not change to the same extent. The second limitation is the method used for measuring color sensitivity in humans. Chromatest is not the gold-standard test for evaluation of color vision. Although quite cumbersome for the patient, electroretinogram may be accepted as the most sophisticated method in this area, and detecting the response to specific colored flash stimuli is theoretically possible. However, in clinical practice it elicits the summed electrical ac-

tivity of all cones in the whole retina (or in a specific region), and good isolation of the response of specific cones especially M and S cones is a difficult task. Chromatest has been reported as an adjunctive tool for the follow-up of adult diabetic patients to document blue-yellow (tritan) color vision and has quite higher sensitivity and specificity compared to Ishihara and higher applicability compared to the Farnsworth-Munsell 100-hue test [17]. Therefore, although Chromatest is not the best way to analyze color sensitivity, it may not be wrong to state that we do not have an ideal method for this measurement yet.

To conclude, as a novel finding, our study has demonstrated that overt hypothyroidism impairs color contrast sensitivity in adults. Interestingly, although significant improvement was seen in color contrast sensitivity after euthyroidism was achieved and red-green color contrast sensitivity was normalized, blue-yellow color contrast sensitivity was still significantly attenuated compared to euthyroid controls in our study. In this regard, the question remains open whether a longer euthyroid period would enable full recovery of color contrast sensitivity. Prospective studies in which evaluation tests are re-performed after a longer euthyroid period may reveal the possibly temporary nature of the TH effect on color contrast sensitivity in adult human subjects.

## Disclosure Statement

None.

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