

Comparative Study of the Impact of Diet Versus Pravastatin on Color Vision in Brodman Area 19 Detected by Computerized Chromatic Analysis (CARDIOCOLOUR Study)

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Introduction and objectives. Hypercholesterolemia causes important neurodegenerative changes in the cerebral cortex, which are manifested by defects in the color perception by the neurons of Brodman area 19. Extensive interventional epidemiological data from both primary and secondary-prevention clinical trials indicate that cardiac ischemic events decrease when total cholesterol or LDL-C is reduced. Our goal was to elucidate the effects of diet compared with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (pravastatin) on color perception using computerized chromatic analysis (CCA) and plasma cholesterol levels.

Patients and methods. We studied 191 normotensive patients (133 men and 58 women) with pre-study plasma cholesterol levels in excess of 200 mg/dl. Seventy of these patients were treated with the American Heart Association Step II diet for six months. The remaining 121 were treated with pravastatin, 61 patients with 10 mg and 60 patients with 40 mg. They were examined by CCA after excluding any general or ophthalmological pathology.

Results. Chromatic vision recovered by 23% with diet, 38% with pravastatin 10 mg and 92% with pravastatin 40 mg.

Conclusions. This study confirmed a strong association between therapeutic intervention with either diet or pravastatin and improved color vision.

Key words: *Test. Cholesterol. Coronary artery disease. Nervous system. Randomized study.*

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Estudio comparativo del impacto de la dieta frente a pravastatina en los centros de análisis cromático del área 19 de Brodman, mediante el análisis computarizado cromático (Estudio CARDIOCOLOUR)

Introducción y objetivos. La hipercolesterolemia origina importantes cambios neurodegenerativos en el córtex cerebral, lo que se traduce en un fallo en la visión cromática, en las neuronas del área 19 de Brodman. Numerosos estudios de intervención terapéutica para la prevención primaria y secundaria de la enfermedad coronaria apoyan una reducción de los acontecimientos cardíacos con la reducción en el colesterol total o el cLDL. Nuestro objetivo fue elucidar los efectos de la dieta y un inhibidor de la HMG-CoA (pravastatina) sobre la visión de los colores, pero también sobre las cifras plasmáticas de colesterol, y correlacionar ambos efectos, mediante el análisis computarizado cromático (ACC).

Pacientes y métodos. Estudiamos a 191 pacientes normotensos (133 varones y 58 mujeres), con valores de colesterol total plasmático por encima de 200 mg/dl. Un total de 70 de estos pacientes fue tratado con la dieta paso II de la American Heart Association durante 6 meses. Los restantes 121 fueron tratados con pravastatina, 61 a dosis de 10 mg y los otros 60 con 40 mg. Fueron examinados mediante ACC, descartando previamente cualquier enfermedad sistémica u oftalmológica.

Resultados. Se logró una recuperación de la visión cromática de hasta el 23% con dieta, del 38% con 10 mg/día de pravastatina y del 92% con 40 mg/día de pravastatina ($p < 0,001$).

Conclusiones. El estudio determina una fuerte asociación entre la intervención terapéutica, con dieta o con pravastatina y la mejoría de la visión cromática.

Palabras clave: *Test. Colesterol. Enfermedad coronaria. Sistema nervioso. Estudio aleatorio.*

INTRODUCTION

The identification of risk factors when evaluating degenerative changes in the cerebral cortex (which leads to a number of types of dementia^{1,2} or cognitive deterioration³ in experimental models) is an important

ABBREVIATIONS

CCA: computerized chromatic analysis.
Db: decibels.

ce advance in the prevention of dementia because identifying mechanisms facilitates the prospect of controlling these types of conditions.⁴ One risk factor that has already been identified as a causes of important neurodegenerative changes is hypercholesterolemia,² which has a genetic origin. Hypercholesterolemia produces, in the central nervous system, a deficit in the cholinergic system that results in a deficit in the molecular biology of the neurons of the cerebral cortex.^{5,6} Hypercholesterolemia also produces deficits in chromatic vision,⁷ which can serve as an early indicator of

cardiovascular risk. Chromatic vision is the highest visual function and occurs only in primates and humans; it can be studied in the associated areas 17, 18, and 19 of the cerebral cortex.⁷ Hypercholesterolemia is one of the primary modifiable risk factors for disease. Numerous observational studies have confirmed the relationship between hypercholesterolemia⁸ and the existence of a continuous and gradual causal relationship between plasma concentrations of cholesterol and death by coronary cardiopathy.^{8,9}

The purpose of this study was to clarify the effects of dietary and pharmacological intervention on the above-mentioned cerebral areas (Figures 1-3), and to extract data from an extensive group of patients without a history of cardiovascular illness, and to establish the significance of dietary and pharmacological

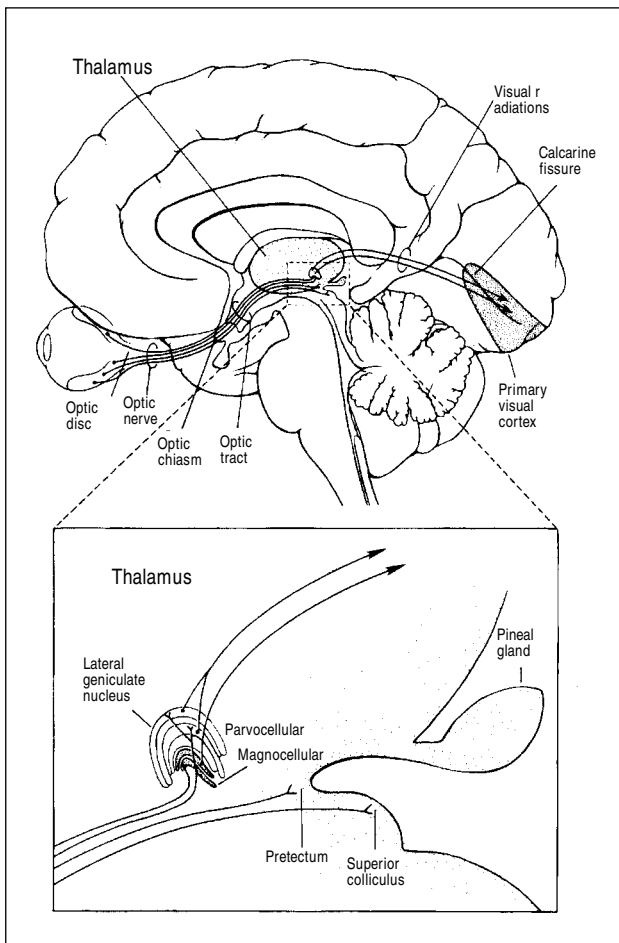


Fig. 1. The graphic depicts a lateral cut in the cerebrum where the total path of the optic pathway is clearly shown from the retina (5% of the pathway) to the cerebral cortex of the Brodman areas 17, 18, and 19. Amplification of the midzone shows the parvocellular tissue (p-channel) responsible for the transmission of color, light, and high sensitivity contrast. Reproduced with the permission of Kandel & Schwartz, from *Principles of Neuroscience* (3rd ed.). Elsevier.

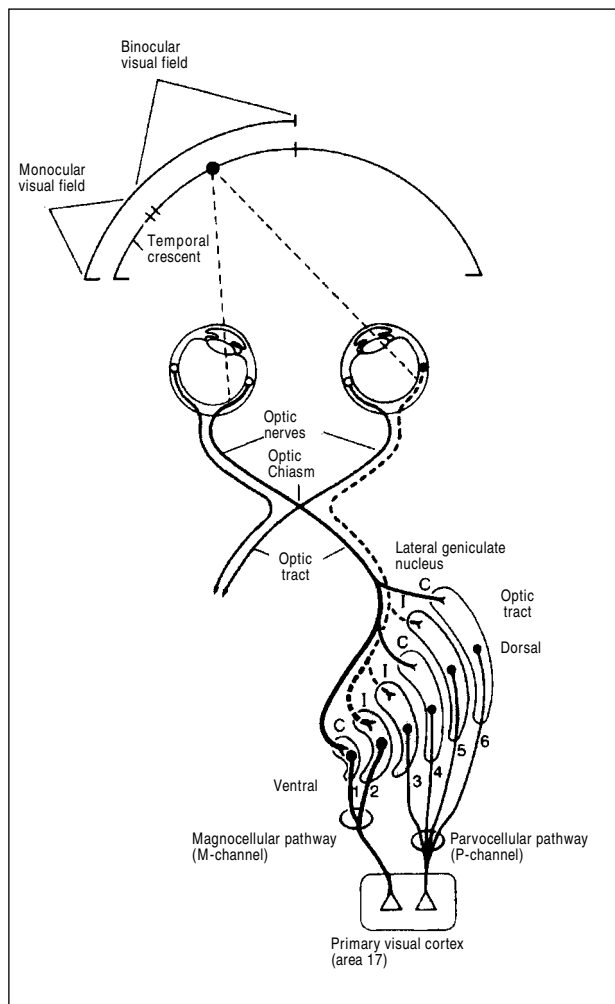


Fig. 2. The graphic depicts an anteroposterior cut seen from above; the synaptic connections over the parvocellular pathway (p-channel) can be seen. The integrity of these connections is susceptible to therapeutic intervention. Improvement of metabolic cellular function results in recuperation of color vision. Reproduced with permission of Kandel & Schwartz, from *Principles of Neuroscience* (3rd ed.). Elsevier.

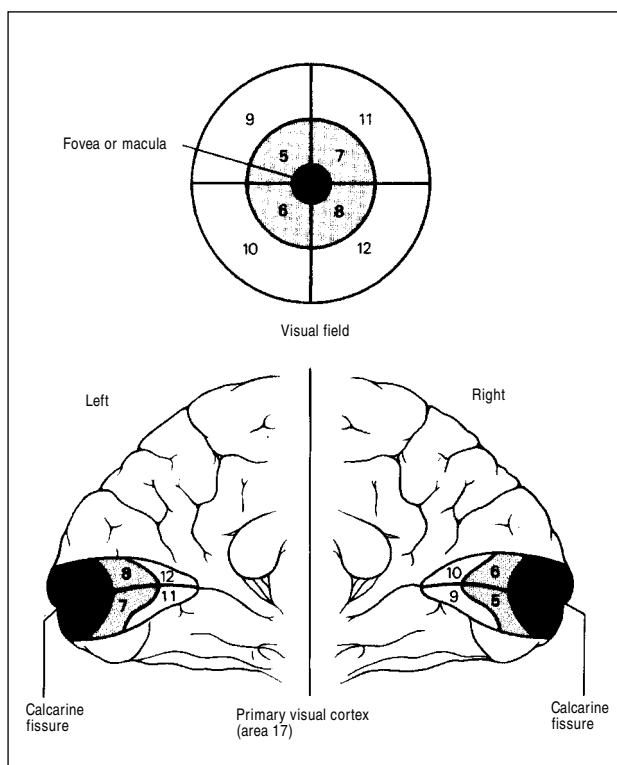


Fig. 3. The graphic depicts a posterior view of the occipital cortex. This area, according to the author, is especially important because, in addition to its principal function (visual), it can act serve as a specific and sensitive indicator of cardiovascular risk associated with hypercholesterolemia. Reproduced with permission of Kandel & Schwartz, from *Principles of Neuroscience* (3rd ed.). Elsevier.

intervention in improving chromatic vision. Verification of substantial improvements in cerebral cortex activity related to color vision allows establishing a definitive correlation between plasma cholesterol values and the cortical neuronal bioelectrical circuits, and also corroborates the neuroprotective role of statins.^{10,11} To this end, we focused our study on the computerized analysis of chromatic vision (CCA)⁷ and studied the minimum perceived saturation of the 4 colors yellow, red, green, and blue separately in each of the subjects studied for each therapeutic intervention (Figure 4). Pravastatin was chosen as the HMG-CoA reductase inhibitor because it is one of the most extensively studied in clinical medicine,⁸ has the least pharmacological interaction, and is the best tolerated.⁹

PATIENTS AND METHODS

This is a consecutive study of 308 patients who signed an informed consent form. Random assignment was made to parallel groups for each of the therapeutic interventions for a period of 6 months. The random assignment process was performed by means of an informatics program administered by the teaching department of the Department of Biostatistics of the Malaga

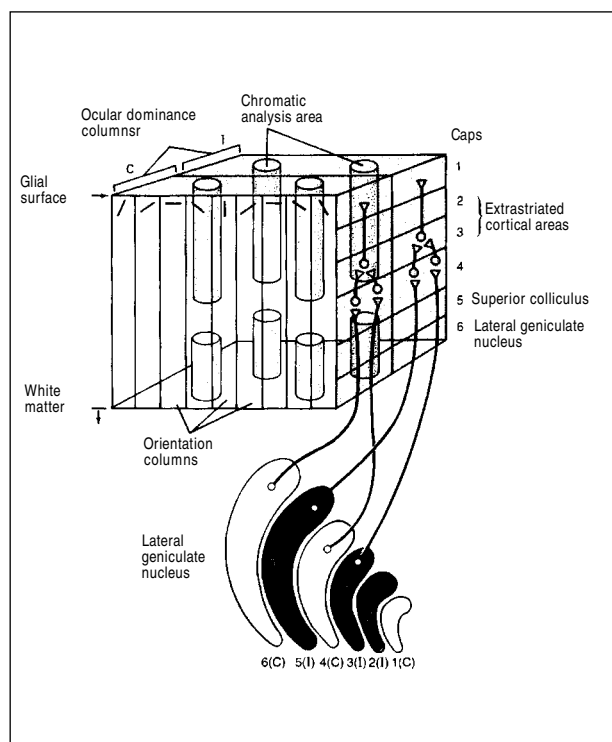


Fig. 4. The graphic depicts the tissular and synaptic structure of the caps of the visual cortex. The structures, called blobs, are the cortical receptor centers of the parvocellular system and analyze color and provide the saturation level at which the subject ultimately sees each of the colors. Reproduced with permission of Kandel & Schwartz, from *Principles of Neuroscience* (3rd ed.). Elsevier.

School of Medicine, whose function is to assign random numbers.

The individuals included in the study were visited at 3-month intervals to verify, on each visit, compliance with the dietetic program or the schedule of pravastatin as prescribed. At each visit a complete physical examination was performed by a physician, and an ophthalmologic examination was performed by an ophthalmologist. During the first 3 months, 85 subjects were excluded from the study, 78 due to limited compliance (92%) and 6 due to concomitant ophthalmologic illnesses. During the second and final 3 months, 32 patients were excluded from the study, 17 due to limited compliance (54%), 8 due to ophthalmologic disease, and 5 for various systemic illnesses. In the group of patients taking 40 mg of pravastatin, 2 patients stopped taking the medication because of adverse effects (one because of myalgia and the other because of abdominal pain). Therefore, between the first and the second 3-month period, a total of 117 subjects were eliminated from the study. Finally, 191 of the 308 initial study participants (133 men and 58 women; age range, 37 to 66 years) passed all the control measures our statistical results were derived from this patient sample.

A total of 70 of the patients were subjected to the step II diet of the American Heart Association (AHA) for 6 months. The diet consisted of reducing total fat to less than 30% of daily calories, consuming 55% more of carbohydrates, 15% or more protein, and consuming less than 200 mg of cholesterol a day. This was the diet followed by the group who were randomized to alimentary-type interventions only.

A total of 61 subjects received treatment with an HMG-CoA reductase inhibitor, pravastatin, at a dose of 10 mg every night after following the step I diet of the AHA for 45 days before initiating treatment.

Finally, 60 subjects were treated with pravastatin at a dose of 40 mg every night for 6 months, after having followed a step I AHA diet for 45 days prior to beginning treatment and thereafter undergoing the treatment.

The step I AHA diet consists of reducing total fat to less than 30% of daily calories, a cholesterol consumption of less than 300 mg/day, sodium consumption of less than 2,400 g/day, carbohydrate consumption of 55% to 60% of total calories, and protein consumption of 10% of total calories.

None of the patients had clinical or other evidence of any respiratory, endocrine, hepatic, renal, or hematological disease. Of the patients, 93.5% were overweight and 6.5% had a body mass index that qualified the patients as obese. Study exclusion criteria were congenital or acquired dyschromatopsia, diabetes mellitus, hyperthyroidism, liver disease, hepatic cholestasis, chest pain or acute myocardial infarct, transitory ischemic accidents, cerebrovascular accidents (CVA), excessive ingestion of alcohol, estrogen hypolipemiant drug treatment, corticoid treatment, immunodepressive treatment, and smoking. Arterial pressure was measured before beginning the study protocol by standard sphygmomanometry techniques after each patient was seated for 5 minutes. Each arterial pressure measurement represents the mathematical average of 3 separate measurements.

Procedures

Clinical biochemical measurements were obtained in accordance with the recommendations of the European Atherosclerosis Society. Blood samples were obtained following a 12-hour period of fasting, after a light supper. Total cholesterol was measured by using the CHOD-PAP (Boehringer Mannheim, Germany) enzymatic technique.¹² Triglycerides were measured by GPO-PAP (Boehringer Mannheim, Germany) enzymatic technique.¹³ Total HDL cholesterol was measured by calcium heparin precipitation (Boehringer Mannheim, Germany).¹⁴ Glucose, creatinine, urea, uric acid, GOT, GPT, and GGT samples were obtained with typical clinical means and analyzed by an automatic analyzer (Hitachi 704, Boehringer Mannheim, Germany).

Ophthalmologic study protocol

First, we performed an exhaustive external inspection of patients who presented with palpebral disease of the ptosis type, prominent eyebrows, or nasal openings that were large enough to produce defects of the superior, inferior, temporal, or nasal visual fields. Afterwards, we examined the pupil^{15,16} to detect afferent or deferent congenital or iatrogenic defects.

After obtaining maximum mydriasis with 1% tropicamide,¹⁷ an indirect binocular Keeler ophthalmoscope was used to examine the central and peripheral retina¹⁷ and the lens was examined with Nikon 90 Dp¹⁸ for a detailed look at the posterior pole.

Any change in the morphology of the optic papilla was noted, as was any retinal vascular change and any parenchyma retina change. The assessment was performed once any vitreous illness was ruled out.¹⁹

The anterior pole was examined with a Haag-Streit BQ 900 slit lamp to detect corneal or crystalline opacities, or any deficit in the anterior pole that could produce a change in lens transparency. Patients with biomicroscopic anomalies were excluded from the study.

Three measurements of intraocular pressure were made in each eye using the Goldman tonometer,¹⁸ and the mathematical was expressed in mm Hg after verifying the instrument precision with 3 additional tonometers of the same type. In the setting of a pressure greater than 21 mm Hg, or less than 21 mm Hg with papillary excavation or campimetric defects, or both, and papillary excavation that could be indicative of a low tension glaucoma,²¹ the patient was excluded from the study.

All of the tests performed considered invalid (or at least of minimal value) if the patient had acquired or congenital dyschromatopsia, or did not have perfectly corrected ametropia if they had the disorder.²² To this end, we excluded all isochromatopsic subjects. In order to correct ametropia, we used a retinoscope, ophthalmometry with Javal keratometer, and refractometry with the Canon automatic. Snellen optotypes were used. The patient was considered to have reached optimal refraction upon achieving a vision unit of ± 0.2 . If the vision reached was less than 0.7 or, for some reason, had a moderate refractory deficit that could affect the tests, whether by refractory scotomata, myopic lesions, or due to angioscotomata typical of hypermetropic patients, the patient was excluded from the study.

In short, any ophthalmologic anomaly resulted in the automatic elimination of the patient from the study, whether due to a palpebral or papillary anomaly, refraction deficits of more than 6, transitory episodes of loss of vision, or as a result of a fundus-copic, biomicroscopic, tonometric, or other anomaly.

TABLE 1. Lipid parameters before and after treatment with increase and decrease expressed as a percentage after intervention (%)

| | Step II AHA diet | | | Pravastatin 10 mg | | | Pravastatin 40 mg | | |
|--------------------------|------------------|-----------------------------|------|-------------------|-----------------------------|------|-------------------|-----------------------------|------|
| | Baseline No. | Intervention No. (% change) | P | Baseline No. | Intervention No. (% change) | P | Baseline No. | Intervention No. (% change) | P |
| Total cholesterol, mg/dL | 255±32 | 227±28 (-11) | .001 | 240±21 | 199±19 (-17) | .001 | 250±30 | 165±15 (-33) | .001 |
| HDL | 53±11 | 57±9 (+7.6) | .001 | 53±11 | 58±5 (+10) | .001 | 52±11 | 56±9 (+9) | .001 |
| LDL | 178±36 | 150±32 (-16) | .001 | 163±30 | 123±28 (-24) | .001 | 173±38 | 88±23 (-49) | .001 |
| Triglycerides | 120±44 | 98±35 (-18) | .001 | 122±36 | 113±36 (-8.5) | .001 | 120±43 | 109±43 (-10) | .001 |

TABLE 2. Initial values from the computerized chromatic analysis before and after therapeutic intervention, expressed in decibels with normalization of the CAA chromatic vision parameters are expressed in %

| | Step II AHA diet | | | Pravastatin 10 mg | | | Pravastatin 40 mg | | |
|--------|------------------|----------------------------|------|-------------------|----------------------------|------|-------------------|----------------------------|------|
| | Baseline No | Intervention No (% change) | P | Baseline No | Intervention No (% change) | P | Baseline No | Intervention No (% change) | P |
| Yellow | 35.1±2.9 | 35.2±2.7 (+23) | .001 | 34.4±3.3 | 36.1±2.6 (+38) | .001 | 34.7±3.1 | 37.9±0.7 (+82) | .001 |
| Red | 25.5±1.6 | 25.2±1.7 (+8.6) | .001 | 23.7±2.7 | 25.3±2.3 (+23) | .001 | 25.1±1.7 | 28±0.6 (+88) | .001 |
| Green | 20.9±1.9 | 21.2±1.8 (+10) | .001 | 19.9±2.6 | 21.9±1.8 (+23) | .001 | 21±1.8 | 24.2±0.6 (+92) | .001 |
| Blue | 21.8±2.2 | 22±2 (+10) | .001 | 19.7±2.9 | 22±2 (+26) | .001 | 21.9±2.2 | 25.2±0.6 (+92) | .001 |

Computerized analysis of chromatic vision

Computerized analysis was performed on the Humphrey 640 computer by Zeiss. This consisted of placing the patient in the examination position, as always with corrected monocular vision, and determining the foveal threshold for each of the colors yellow, red, green, and blue.⁷ Red, green, and blue could be tested automatically by the Humphrey 640, and to test yellow we used white light with a Cibachrome Y II filter in place.⁷

The values for each color were expressed in decibels (db). The standard colors found were of 38, 28, 24, and 25 for the colors yellow, red, green, and blue.⁷ The test was performed on both eyes of each patient, but only the measurement of the second eye was used in order to control for a possible learning effect of the test.

Statistical analysis

We performed a separate study analyzing, on one hand, the patients who only followed a diet and, on the other hand, patients to whom pravastatin was administered. In each case, to verify the possible significant differences between the values for each patient at baseline (baseline = before beginning the diet or starting pravastatin treatment) and after diet or treatment (to evaluate the efficacy of both treatment methods) we used the parametric Student *t* test for paired data in each of the parameters studied.

In a second analysis of each treatment, we classified subjects according to what was considered a normal or

abnormal value for the patient for each parameter.⁷ In order to evaluate the patients who normalized in each of the 4 groups studied, we performed the McNemar test for paired data. This last analysis was verified by the 2-proportion contrast method.

RESULTS

Table 1 shows the 3 groups of patients studied and their lipid characteristics at the beginning of the study. All the groups had total cholesterol of more than 200 mg. The effects of the therapeutic intervention in the 3 groups with the percentages of increase or decrease in lipid values is shown. The group with the step II AHA diet achieved, after intervention, an approximate 11% decrease in total cholesterol and a 16% decrease in LDL cholesterol. The group that took 10 mg of pravastatin reduced their total cholesterol by 17% and their LDL cholesterol by 24%. The group that took 40 mg of pravastatin showed a marked difference from the other groups, with a 34% decrease in total cholesterol and a 49% decrease in LDL cholesterol.

Table 2 shows the values from the computerized chromatic analysis before and after therapeutic intervention, expressed in db for each of the 3 groups, step II AHA diet, pravastatin 10 mg, and pravastatin 40 mg. We included the normalization of CAA chromatic vision results in percentages. The group following the step II AHA diet achieved between a 10% and a 23% improvement. The group taking pravastatin 10 mg achieved between a 26% and 38% improvement, and, finally, the group taking 40 mg of pravastatin and fo-

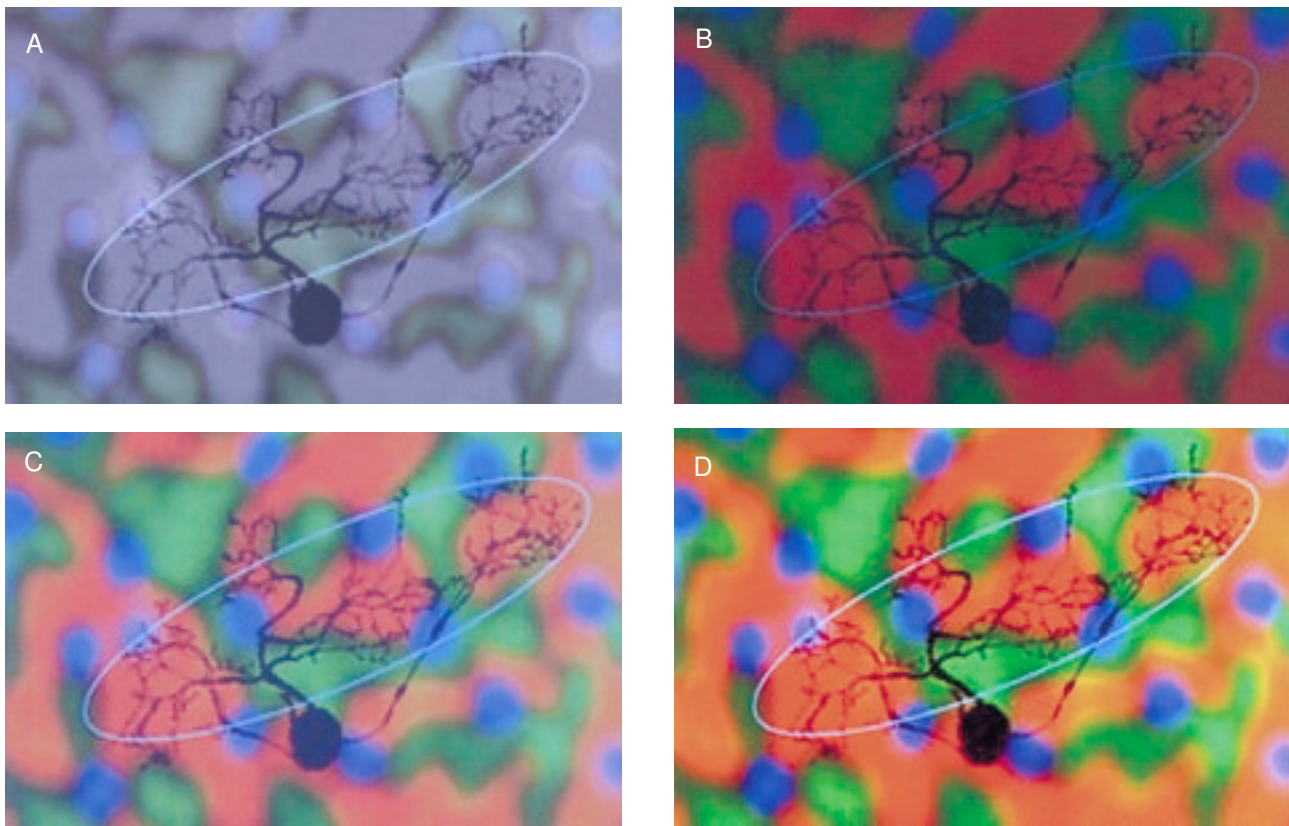


Fig. 5. Photographic montage of a cortical neuron during visualization by a patient in different situations. On the upper left is an image corresponding to that visualized by a patient with a total cholesterol of >230 mg/dL (at the beginning of the therapeutic intervention in this study). The low color saturation perceived by this patient can be seen. On the upper right is an image as would be visualized a patient after 6 months of following the AHA step II diet. Better color saturation can be observed, as much as that correctly perceived in Brodman area 19. On the lower left is an image as visualized by a patient treated with 10 mg/day of pravastatin for 6 months. Increased color saturation can already be seen in tenths of db, sufficient to perceive with greater correction chromatic vision in Brodman area 19. On the lower right is an image as visualized by a patient treated with 40 mg/day of pravastatin for 6 months in addition to following the AHA step I diet. Excellent chromatic saturation can be seen, increased by 92%, allowing almost completely normal color vision in Brodman area 19.

Following the step I AHA diet had an improvement in their chromatic vision parameters of up to 92% (Figure 5).

DISCUSSION

There are many patterns of neuronal lesions of the optic pathway that produce a change in chromatic vision. The traditional known list of causes such as trauma,²³ destruction due to pressure,²⁴⁻²⁶ or metabolic causes²⁷ has recently been augmented by the addition of distinct models of toxic neuronal lesion due to overstimulation of the plaque aggregation factor (PAF)²⁸ by direct action of nitric oxide,²⁹ including lipid peroxidation,³⁰ as a result of the liberation of free radicals. But there is also proof that hypercholesterolemia is a neurotoxic and neurodegenerative factor that produces changes in chromatic vision.⁷ This alteration of chromatic function does not only indicate the existence of hyperlipemia, but also indicates changes in the cellular

membranes that results in deficits in the molecular biology of the cerebral cortex areas 17, 18, and 19 (Figure 1),^{31,32} which predicts early (using computerized methods) a cardiovascular risk based on plasma values of total and LDL cholesterol (Figure 5A). Firstly, the direct relationship between the plasma values and the neurons of the visual cortex⁷ has been adequately demonstrated in our study of 191 patients, corroborating previous results.⁷ There is a mechanism of action that has a toxic effect on the neurobiology of that cerebral area that is affected primarily by hyperlipemia that results in a quantifiable loss of chromatic vision loss that ultimately affects the P23 cells (Figure 2) of Brodman areas 17, 18, and 19 evident on chromatic analysis³⁴ (Figures 2-4). Secondly, we have shown the previously unreported finding that the response of neurons to the lipid profile varied significantly according to the therapeutic intervention prescribed; the results achieved in terms of plasma cholesterol values were not parallel to the lipid value results. Therefore, in the

group who followed the AHA step II diet, the decrease in total cholesterol was 11% and the decrease in LDL cholesterol was 16%, decreases that vary from the documented improvement in chromatic vision, which varied from 10% to 23% (Figure 5B). In the group taking 10 mg of pravastatin, the decrease in total cholesterol was 17% and the decrease in LDL cholesterol was 24%, decreases which vary from the documented improvement in chromatic vision, which ranged from 26% to 38% (Figure 5C). Finally, in the group taking 40 mg of pravastatin and following the AHA step I diet, the decrease in total cholesterol was 33% and in LDL cholesterol was 49%, decreases that were at variance with the documented improvement in chromatic vision, which ranged from 82% to 92% (Figure 5D). Therefore, we have documented improvement certain neuronal plasticity, and a collateral effect, in addition to a simple lowering of lipid values,⁹ the supposed neuroprotective effect of HMG-CoA reductase inhibitors (which seems, according to our study, to be dose-dependent). In any case, it is notable that a multicenter prospective study appears to show that the mg dose of statins—prescribed according to the standard procedures of clinical cardiology—appears to be related to the results obtained directly obtained from neuronal tissue as described in this study. All researchers are charged with being able to read and interpret the facts in clinical medicine; here we only provide a minimal degree of proof that a tissue exists that not only serves to produce the physical sensation of vision but also possesses its own indicators that we must be able to read. On the other hand, and according to the data presented in our study, we have an excellent pharmacological tool for recovering chromatic function, an effect that was previously unknown, along with the collateral beneficial effects of taking statins.³⁵ Additionally, this study supports the view that patients with hypercholesterolemia do not have good chromatic vision,⁷ and that with CAA we can analyze the blue-yellow axis and find out whether the mechanism of action was pre-existing or newly developed;³⁴ the technique, in the form of blue-yellow perimetry, has been used since 1996 for the early diagnosis of glaucoma.³⁷ This point may be very interesting for the clinical management of patients. Thirdly and lastly, epidemiological studies^{8,9,38,39} have convincingly proven that higher plasma cholesterol values mean a higher risk of heart disease. These studies have shown a decrease in heart disease when total and LDL cholesterol values are decreased.⁷ The effective control of hyperlipemias is currently universally accepted as a useful tool for the preventing cardiovascular disease. Other studies have shown that identifying and acting on lowering cholesterol values can save lives.^{8,39,40}

There are many very sensitive mechanisms that regulate intracellular cholesterol concentrations. It is possible that recovering biological functions as specia-

lized as color vision in humans requires very specific decreases in cholesterol levels that result from pharmacological therapeutic intervention. If the areas chromatic vision analyzed affect what statin dose should be used (based on improving cellular biology in these areas up to 100% of capacity) the clinical management of patients could incorporate, perhaps, a new paradigm of pharmacological treatment that is based on information gleaned from the most specialized of tissue, nerve tissue, and the most specialized group of cells within that tissue, cortical neurons. The dose that we tried of 40 mg of pravastatin which resulted in recovery of chromatic vision is, curiously, the same dose that in the WOS study⁸ was shown to reduce the risk of fatal or nonfatal coronary events by 30%.

The usefulness of these findings is double for ophthalmologic applications. On the one hand, the results are useful for the identification and treatment of acquired dyschromatopsia induced by hypercholesterolemia and, on the other hand, may be an indirect key to the prevention of the most frequent cause of death in the developed world.⁴⁰

CONCLUSIONS AND CLINICAL IMPLICATIONS

1. Our study establishes a strong association between therapeutic intervention, either with diet or pravastatin, and improvement in chromatic vision.
2. Pravastatin has a collateral effect, by direct or indirect mechanisms, of recovering 92% of color vision, which was unknown until the current study results.
3. The neuroprotective effect of statins appears to be corroborated in Brodman area 19.

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