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The acute effect of cocoa and red-berries on visual acuity and cone-mediated dark adaptation in healthy eyes

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ABSTRACT

The retina is a highly vascularized tissue with a high metabolic and oxygen demand responsible for human vision. Considering that the polyphenolic flavanols and anthocyanins have been shown to be beneficial for endothelial function and cerebral blood-flow, an acute randomized and controlled crossover trial with two different sources of polyphenols, anthocyanins from red-berries and flavanols from cocoa, was designed to better understand the effect of polyphenols on visual acuity (VA) and cone-mediated dark adaptation (DA). Thirty-seven healthy subjects (22.1 ± 2.0 years old) participated in the acute intervention for three times (red-berries, cocoa or vehicle-control) with a washout period of two weeks in-between. VA under photopic and low luminance (mesopic) conditions, DA or dynamic of recovery of contrast threshold (CT) following near-total photopigment bleach for 5 min, urine total polyphenols, theobromine and antioxidant power were measured in the three study-arms after 2-hours ingestion of the study-food. 3-hours postprandial urine showed higher levels of total polyphenols after ingestion of cocoa flavanols or red-berries anthocyanins in comparison with the vehicle-control and higher levels of theobromine only for the cocoa group. There was an increase in photopic VA with cocoa flavanols that with red-berries anthocyanins did not reach statistical significance. Both, cocoa and red berries, failed to improve mesopic VA and the cone time constant for contrast recovery and final CT of DA.

1. Introduction

Polyphenols are non-nutrient food components with proven antioxidant, anti-inflammatory and vasoactive properties (de Pascual-Teresa, Moreno, & García-Viguera, 2010). These properties of polyphenols have been the main basis for their use in eye-active food supplement formulation and their clinical use in the last years (Rossino & Casini, 2019). The physiology of vision and eye health are influenced by dietary factors at various levels. Part of those factors influence blood flow and blood pressure at the eye level which will utterly affect visual function. Moreover, dietary factors may affect the visual signal at transduction level, either in the activation of rhodopsin by the action of light or on its regeneration to return to its initial configuration.

Cocoa and red-berries are good sources of polyphenols in human diet (de Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000). Red-berries are characterized by the presence of anthocyanins, a group of red-purple-blue flavonoids that have been associated with endothelial protection and inflammation. Cocoa is rich in flavan-3-ols but also contains other polyphenols such as phenolic acids and flavonols. Additionally, cocoa is a good source of the methylxanthine theobromine, which in contrast with caffeine has a lesser psychoactive effect but can reduce blood pressure while caffeine has shown to increase it depending on the person (Baggott et al., 2013; Mitchell et al., 2011).

Cocoa and red-berry polyphenols have been associated with the protection against cardiovascular and neurological conditions in numerous recent studies (de Pascual-Teresa et al., 2010). Both processes are directly or indirectly involved in visual function and eye health and might justify some of the results showing an association between polyphenols consumption and an improvement in visual health. In addition, vision is strongly affected by oxidative processes (the retina is a highly vascularized area with a large amount of unsaturated fatty acids), therefore those components of the diet capable of reaching that area of the organism could protect the retina against such oxidation. In this sense, anthocyanins can become localized in ocular tissues of rats, rabbits (Matsumoto, Nakamura, Iida, Ito, & Ohguro, 2006) and pigs (Kalt, Hanneken, Milbury, & Tremblay, 2010). Other polyphenols, such as

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epicatechin or epicatechin-3O-gallate have shown to cross the Blood-retinal-barrier (BRB) and thus they have the potential of acting at the eye level (Liu et al., 2017). On the other hand, anthocyanins have shown to protect retinal cells from UVA light induced damage in in vitro conditions (Silván, Reguero, & de Pascual-Teresa, 2016).

Some previous studies have shown a potential for polyphenols on eye health. Although anthocyanin intake was associated for a long time with an improvement on night vision in healthy individuals most of the early clinical research that reported effects of anthocyanin on night vision did not employ a randomized placebo-controlled design. Conversely, later reports demonstrated that anthocyanins given in single or multiple oral doses had no effect on night vision in healthy eyes (Canter & Ernst, 2004; Kalt, McDonald, Fillmore, & Tremblay, 2014). In myopia subjects with asthenopia, the treatment with purified high-dose anthocyanins (85 mg) appears to improve subjective visual symptoms and contrast sensitivity measured under low luminance conditions (mesopic conditions) (Lee et al., 2005).

Other type of polyphenolic flavonoids, such as catechins and proanthocyanidins from grape seeds, have shown to improve visual adaptation to low luminance and in general visual performance (Corbé, Boissin, & Siou, 1988) and have largely proven an antioxidant and vasorelaxant effect at different tissue levels. In healthy subjects, flavanol-rich cocoa induces peripheral vasodilation via activation of the nitric oxide system (Fisher, Hughes, Gerhard-Herman, & Hollenberg, 2003). Cocoa flavanols improved spatial attention, reflected by reduced reaction time (Karabay, Saija, Field, & Akyürek, 2018). The effect of cocoa flavanols on visual function has been little explored. Recently, short-term improvement in small-letter or number contrast sensitivity (Field, Williams, & Butler, 2011) and high-contrast visual acuity (Rabin, Karunathilake, & Patrizi, 2018) has been observed after consumption of dark chocolate. Conversely, no short-term effects of flavanol-rich dark chocolate on automatically assessed retinal blood flow on optical coherence tomography (OCT) angiography (vessel density) or subjective visual function (visual acuity and large-letter contrast sensitivity) were observed (Siedlecki et al., 2019). To our knowledge, the effect of flavanols on night vision has not yet been explored.

Night vision can be assessed by measuring the retinal dark adaptation (DA), a highly sensitive neural function. DA is the slow recovery of sensitivity to a luminance target following exposure to a light that bleaches the photopigments of the photoreceptors. The rate of recovery or speed of retinal DA is dependent on the regeneration of photopigments and therefore on the transport of nutritional support and/or metabolic supply to the outer retina (Lamb & Pugh, 2004). The time course of the recovery function is divided into rod- and cone-mediated sections (Lamb & Pugh, 2004). While the effect of anthocyanins on rod-mediated DA has been the focus of most studies (Canter & Ernst, 2004; Kalt et al., 2014) the effect of polyphenols (anthocyanins and flavanols) on cone-mediated DA has not yet been addressed.

To establish whether the effect of polyphenols on vasodilatation is translated into better visual function under low luminance conditions, when the oxygen consumption in the retina increases we aimed to study the acute effect of cocoa flavanols and red-berries anthocyanins on retinal functions such as visual acuity (VA) and cone-mediated dark adaptation (DA) in healthy eyes. Additionally, we aimed to bring out a possible correlation between polyphenols or methylxantines in 3-hours urine and visual function.

2. Materials and methods

2.1. Subjects and study design

This was a randomized cross-over study, in which the effects of cocoa, red-berries and a control were assayed in three single acute doses. The research project was approved by the Ethics Committee of the San Carlos Clinic Hospital (C.I. 18/510-TFM_R_X) and by the Bioethics Committee of CSIC (141/2018). All subjects signed a written informed consent prior to their participation in the study.

Inclusion criteria were a corrected distance VA of 0.9 decimal or better in at least one eye, a refractive error not greater than ± 4.50 dioptres (D) sphere or ± 1.75 D cylinder and a normal ophthalmologic examination. Exclusion criteria were body mass index (BMI) <18 or >30 Kg/m^2, chronic or current medical treatment, antibiotic treatment in the previous two months and consumption of polyphenol containing food supplements. 40 young subjects were recruited, of which 37 (20 men and 17 women 18 to 27 years old) finished it. In each subject, the right eye was selected unless it did not meet the inclusion criteria, in which case the left eye was included.

The participants attended the Faculty of Optics and Optometry in three different visits where they consumed a drink with cocoa, a drink with red-berries and a vehicle-control (milk). A reduced-fat milk was chosen as a vehicle for the cocoa flavanols and red-berry anthocyanins as well as a control in this study due to its fat, protein and water contents and its general acceptance in polyphenols intervention studies. Before every visit, participants were asked to refrain from eating or drinking any polyphenol or caffeine rich food or drink for the previous 24 h. Participants were given a list of items that they should no consume in order to help them meet this requirement. Participants were randomized in three groups to start the study in a different study-arm (cocoa, red-berries or control) with at least two weeks wash-out period. Outcomes were measured in the three study-arms after 2-hours ingestion of the study-food.

Pure semi-defatted cocoa from organic farming, with high polyphenols contents (7 g/100 g cocoa powder) and a freeze-dried mixture of red-berries mixture containing 33.3% raspberries, 33.3% black-currant, 16.7% red-currant and 16.7% blueberries powder (1.7 g/100 g red-berries powder), were commercial products and were kindly supplied by Salengei® (Barcelona, Spain). The daily amount of cocoa (2.5 g) and red fruits powder (10 g) was homogenized to be 175 mg total polyphenols in these two study arms, and zero for the control diet. Total flavanol contents in cocoa was 1.5 mg/g and total anthocyanin contents in the red-berry powder was 3.2 mg/g (for methodology see Silván et al., 2016). The composition of the diets is detailed in Table 1.

Twenty-four-hour diet recall questionnaires were completed by each participant with the aid of trained interviewers on each study arm. The interviews were done in all the three periods of the study in order to stablish possible changes in total energy and nutrients intake that might have influenced the final result of the present study. Data collected on the foods and amounts consumed were introduced into a software application in order to calculate nutritional intakes of macro and micronutrients (DIAL®, Alce Ingenieria).

2.2. Total polyphenols and antioxidant analysis

Total polyphenols (TP) were measured in urine samples collected at least 2 h after ingestion of the assayed food. Total polyphenols were

Table 1
Total nutritional composition of the control, cocoa and red fruit diets per serving*

	Control diet	Cocoa diet	Red Fruit diet
Energy (Kcal)	125.01	142,66	160.56
Total fat (g)	3.56	4.11	3.83
Saturated fat (g)	1.90	2.24	1.91
Carbohydrates (g)	18.57	19.57	27.22
Sugars (g)	9.04	9.17	12.58
Fibre (g)	0.38	1.98	4.37
Proteins (g)	4.54	5.93	5.27
Salt (g)	3.30	3.31	3.32
Total Polyphenols (mg)	0.00	184.42	169.50
Methylxanthynes (mg)	0	45.18	0
Theobromine (mg)	0	38.69	0

*Data are expressed as the amount of each nutrient contained in the full meal consumed in each study-day.

analyzed by the Fast-Blue method (Lester, Lewers, Medina, & Saftner, 2012) that was modified for its application to urine samples. Briefly, 150 μL diluted urine (1:4 in water) were placed in a 96-well plate well, and the reaction was started by adding 15 μL/well of 0.01% Fast Blue in water and 15 μL/well of 5% NaOH. After incubating the plate for 120 min at room temperature, the absorbance was recorded at 420 nm in a BioTek Synergy HT multi-mode microplate reader with BioTek's Gen5TM software (BioTek Instruments Inc., Winooski, VT, USA). Data were expressed as gallic acid equivalents.

In order to normalize total polyphenols data, creatinine in urine was determined by a colorimetric reaction (Roura, Andrés-Lacueva, Estruch, & Lamuela-Raventós, 2006). The method was modified for its determination in 96-well plates. To do this, 10 μL urine diluted samples (x20) were pipetted into each 96-well flat bottom plate well in triplicate. Subsequently, 200 μL 0.1% picric acid and 15 μL 5% NaOH were added to each well. After incubation for 15 min at room temperature and in dark conditions, absorbance at 500 nm was recorded in a plate reader (BioTek Synergy HT multi-mode microplate reader). Data were expressed as gallic adic equivalents in g per g of creatinine in urine (TPC).

2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) is a stable radical widely used to monitor the free radical scavenging ability (the ability of a compound to donate an electron) of a given sample or antioxidant compound. The DPPH reducing ability of plasma assay was performed as previously described. The DPPH method measures the capacity of, in this case, antioxidants in human urine, to donate a hydrogen and reduce the DPPH radical. Briefly, 10 μL urine was placed in a 96-well microplate well (in triplicate), and 290 μL DPPH reagent was added. After 1-hour incubation at 37 °C on a rotary shaker, the absorbance was measured at 517 nm. DPPH values were expressed as Trolox equivalents in g, per g creatinine in urine (DDPHC) in the same way as for total polyphenols.

2.3. Methylxanthines in urine

Methylxanthines in urine were measured by a modified version of the method by de Aragão, Veloso, Bispo, Ferreira, and de Andrade (2005). Briefly, urine samples were kept at −80 °C until analyses. 0.5 ml urine were mixed with 0.5 ml ethanol (25% in water), vortexed for 5 min and centrifuged for 15 min at 12,000*g*. The supernatant was collected and samples were filtered throw a 0.45 μm nylon filter and transferred into HPLC vials. For HPLC-DAD analysis an Eclipse XDB-C18 (5 μm, 4.5 × 150 mm) Agilent's column was used with isocratic 10% acetonitrile in formic acid 0.5% in water at a flow rate of 0.8 ml/min. Standard curves for caffeine, theophylline and theobromine were constructed between 0.5 and 100 μmol/L showing good linearity in the range of concentrations essayed and with limits of detection bellow 1 μmol/L for all three methylxanthines. Additionally, a blank and a known concentration of the three standards was injected every 6 samples to avoid changes in retention time and accumulations in the column.

2.4. Visual acuity measurements

Monocular corrected-distance VA was measured using high-contrast Bailey-Lovie logMAR letter charts under photopic (100 cd/m^2) and mesopic (0.1 cd/m^2) luminance conditions at a distance of 4 m. Measures of luminance for the tests were obtained using a MAVO-SPOT 2 USB luminance meter (Gossen Lighting Control, Nuremberg, Germany). First, VA was measured under the maximum room lighting conditions. Second, VA was measured in the dark with the chart externally illuminated with a halogen lamp that allowed the adequate mesopic luminance level (Puell et al., 2012). The subject was dark adapted during 5 min before mesopic VA measurement was carried out. All VA were expressed as the logarithm of the minimum angle of resolution (logMAR). Each letter read correctly on each line was given a score of 0.02 log units. A loss of one line of letters corresponds to a logMAR increase of 0.1.

2.5. Dark adaptation measurements

Cone-mediated dark adaptation (DA) was measured using a previously described method (Puell & Fernandez-Balbuena, 2019) based on contrast detection instead of luminance detection to study sensitivity recovery not only at prereceptoral stage but also at post-receptoral processing, since visual contrast mechanisms are mediated through inner retinal cells (Lee, 2011). Briefly, the recovery of spatial contrast sensitivity (inverse of contrast threshold) following a photobleach was measured within the central retina for 5 min in darkness. A subject's threshold for detection of a sine-wave grating's orientation (oriented either horizontally or vertically) was determined for a stimulus of low spatial frequency (1 cycle per degree, cpd) and low mean luminance (1 cd/m^2). The gratings generated in a circular patch subtended 5° at a viewing distance of 1 m. Monocular contrast thresholds were measured for 5 min immediately after the subject's retinal bleach using a modified staircase procedure. On correct responses, the contrast of the grating was decreased 0.30 log units of its previous value. Incorrect or absent responses resulted in an increase of 0.15 log units of the previous grating's contrast value. Contrast threshold and time were recorded when the stimulus orientation was correctly identified on an ascending staircase. Contrast was specified as Michelson contrast: $([L_{max} - L_{min}]/[L_{max} + L_{min}])$ where L_{max} and L_{min} represent the maximum and minimum luminance in a grating, respectively.

Gratings were displayed on the center of a calibrated gamma-corrected high-resolution CRT monitor with a mean background luminance of 10 cd/m^2 under dark-room conditions. The mean screen luminance was reduced from 10 cd/m^2 to 1 cd/m^2 (mesopic light level) using a neutral density filter. Luminance level was measured with a light meter (MAVO-SPOT 2 USB light meter, Gossen Lighting Control, Nuremberg, Germany). An electronic 0.9 ms photographic flash of white light (Speedlight SB-800, Nikon, Japan) was used to induce a retinal bleach.

Before data collection, all subjects undertook practice runs. A chin and head rest was used to maintain fixation and view the screen monocularly while wearing best spectacle correction if needed.

Fig. 1 shows an example of a dark adaptation curve, plotted as $\log_{10}$ contrast threshold versus time in seconds, following a retinal bleach for a subject from the study sample. Contrast threshold recovery functions

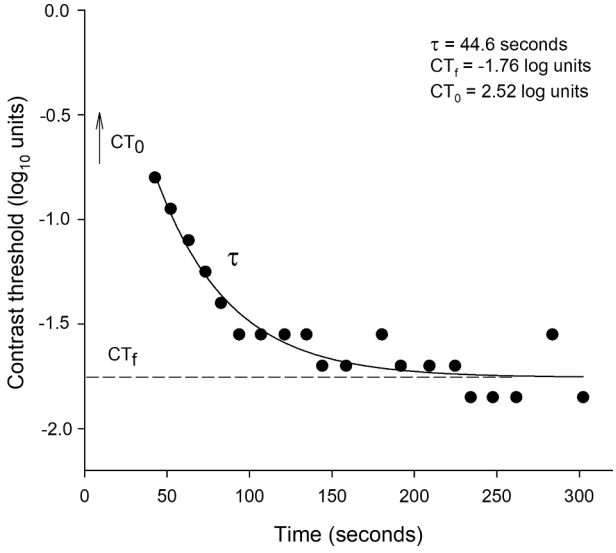

Fig. 1. Contrast sensitivity recovery following retinal bleach as a function of time in darkness for a subject from the study sample measured after a single acute dose of cocoa The solid curve is the best-fit single exponential decay model. t: cone time constant for contrast recovery, CT_f: final contrast threshold, CT_0: contrast threshold elevation at time zero.

were analysed by non-linear regression to determine dark adaptation parameters using SigmaPlot 11 software (Systat Software Inc). Data were fitted to a three-parameter single exponential decay equation:

$$C(t) = CT_f + CT_0 \cdot e^{-t/\tau} \quad (1)$$

where C(t) is the contrast threshold at time t after the bleach, CT_f is the final or absolute contrast threshold (log_{10} units), CT_0 is the contrast threshold elevation above the absolute threshold at time zero (log_{10} units) and τ is the time constant for contrast recovery (seconds).

2.6. Pupil sizes measurements

Pupil size was measured to control for the influence of retinal illumination on visual function. It is well known that smaller pupils decrease retinal illumination. Pupil sizes were measured using a Colvard infrared pupillometer (Oasis Medical, Glendora, California) first at the photopic light level and second at mesopic light level after the subject had spent at least five min in total darkness to ensure adequate dilatation. All subjects had pupils greater or equal to 4 mm in diameter.

2.7. Statistical analysis

According to prior power calculations, for a critical P value of 0.05 the minimum sample size was 37 subjects per diet group. This would be sufficient to detect statistical significance for an anticipated mean time constant difference of 17 s between the intervention groups. The calculation assumed an overall variability of 22 s and a power of 0.90.

The results were reported as means ± standard deviation (SD) performed in triplicate. First we checked for data normality by using Kolmogorov-Smirnov All data showed a normal distribution but pupil sizes. In this last case, we used a multinomial design after transformation of the data (data ≤4.9 or >5.0). All other data was analysed by a mixed error-component model with a post hoc Bonferroni test. Differences were considered significant at $p < 0.05$. All statistical tests were performed with IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

3. Results

A total of 40 subjects were recruited and started the study. Three of them did not finish the study due to agenda problems mainly. From the 37 volunteers who finished the study, 17 were women and 20 men with an age between 18 and 27 years old (22.1 ± 2.0 y), a BMI of 23.4 ± 2.4 Kg/m^2 (18.6–27.7 Kg/m^2) and a waist to height ratio of 0.49 ± 0.04 cm/cm. Mean spherical equivalent was −0.72 ± 1.30 (D). No difference was shown in total energy or nutrient intake between study days for all subjects after analysis of data from 24-h dietary recall. No effect of sequence was shown for any of the parameters analysed.

Table 2 provides the theobromine, total polyphenols/creatinine ratio (TPC) and antiradical activity (DPPHC) data obtained in urine samples after consumption of the cocoa, red-berries and control diet. There was a main effect of the diet for mean theobromine in urine ($p < 0.001$). Cocoa diet urine theobromine was increased by 36.22 μmol/L in comparison with that of red-berries diet ($p = 0.005$) and by 45.95 μmol/L ($p = 0.000$) related to the control diet. Total polyphenols (TP) in urine was 41.27 μg/mL higher after red-berries ($p = 0.034$) and did not reach statistical significance after cocoa consumption ($p = 0.456$) in relation with the control diet (main effect of the diet $p = 0.036$). When TP data were normalized with total creatinine data in urine (TPC), there was a main effect of the diet for mean TPC ($p = 0.002$). Mean total polyphenol/creatinine ratio (TPC) was 44.36 μg/mg higher for the red-fruit diet than for the control ($p = 0.004$) and a 29.03 μg/mg higher TPC for the cocoa diet ($p = 0.034$). No significant differences were observed for TPC between the cocoa and red-berries diet. When stratified by sex a similar trend was shown for both sexes, however there was a significant trend to a higher TPC for every diet in the case of females ($p < 0.001$) than that for males but there was in both sexes a significant increase of TPC after cocoa and red berries against the control diet (Fig. 2). No significant differences (main effect of the diet $p = 0.661$) were observed between the diet groups in the mean values obtained for antioxidant power expressed as DPPH as Trolox equivalents in g and normalized per g creatinine in urine DPPHC).

Table 2
Theobromine, total polyphenols and antiradical activity in urine. Mean ± SD (min, max).

	Control	Cocoa	Red-berries
Theobromine (μmol/L)	5.18 ± 1.50 (0.00; 29.97)	51.13 ± 8.09** (0.00; 174.19)	14.91 ± 7.13 (0.00; 77.74)
TPC (g eq gallic adic/g creatinine)	94.3 ± 39.7 (28.8; 191.8)	123.3 ± 53.0* (40.8; 250.0)	138.6 ± 69.0* (37.7; 291.9)
DPPHC (μmol eq Trolox/ g creatinine)	113.9 ± 49.2 (34.4; 218.3)	116.3 ± 71.5 (43.0; 231.4)	119.1 ± 66.4 (23.8; 306.8)

TPC: urine total polyphenols normalized with creatinine, DPPHC: antioxidant activity as DPPH value referred to creatinine. One asterisk indicates significant increase compared to vehicle-control treatment with $p < 0.05$; two asterisks with $p < 0.01$.

Table 3 provides the mean values of photopic and mesopic VA and pupil size and mean dark adaptation parameters recorded in the cocoa, red-berries and control diet groups.

The mixed error-component model showed a main effect of the diet for mean photopic VA ($p = 0.034$). The post hoc Bonferroni test found that mean photopic VA was 0.04 logMAR (2 letters on the chart) better in the cocoa group than in the control diet group ($p = 0.032$) while was only 0.03 logMAR (nearly 2 letters of VA) better ($p = 0.064$) in the red-berries diet group than the control diet group but failed to reach statistical significance. Mean photopic VA measurements made under the three diet conditions did not differ in the female and male groups and there was no interaction ($p = 0.930$) (Fig. 3). Means for mesopic VA, photopic and mesopic pupil size and dark adaptation parameters (cone τ, CT_0, CT_f) did not vary significantly between the three diet groups.

4. Discussion

This study was designed to investigate the effect of acute cocoa and red-berries ingestion on visual acuity and cone-mediated dark adaptation in young adults with a randomized, counterbalanced, crossover design. Our main finding was that photopic VA improved significantly after cocoa ingestion but only showed a trend to an improvement with red-berries ($p = 0.064$). Mesopic VA and dark adaptation were not

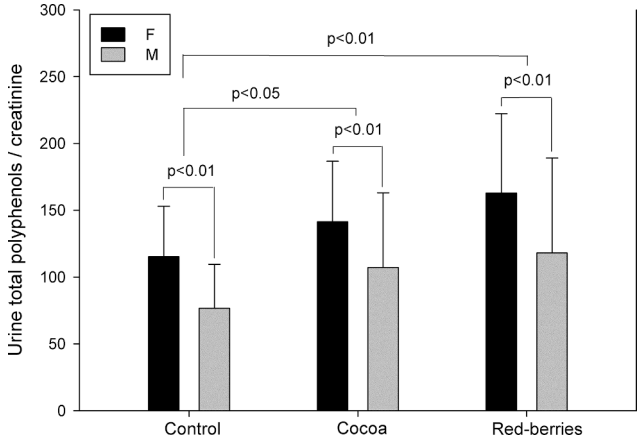

Fig. 2. Mean total polyphenols/creatinine ratio measurements made under the three diet conditions (control, cocoa and red-berries) in the female and male groups.

Table 3
Visual acuity, pupil size and cone-mediated dark adaptation parameters. Mean ± SD (min, max).

	Control	Cocoa	Red-berries
Photopic VA (logMAR)	−0.07 ± 0.07 (−0.22; 0.06)	−0.11 ± 0.05* (−0.22; −0.04)	−0.10 ± 0.07 (−0.26; 0.06)
Mesopic VA (logMAR)	0.37 ± 0.11 (0.18; 0.60)	0.33 ± 0.12 (0.12; 0.60)	0.34 ± 0.10 (0.18; 0.56)
Photopic pupil size (mm)	4.28 ± 0.45 (4.00; 5.00)	4.46 ± 0.53 (4.00; 5.50)	4.60 ± 0.66 (4.00; 6.00)
Mesopic pupil size (mm)	5.96 ± 0.84 (5.00; 8.00)	6.00 ± 0.91 (4.50; 8.00)	5.89 ± 0.71 (5.00; 7.50)
Cone τ (seconds)	55.9 ± 21.4 (26.6; 107.0)	58.2 ± 20.9 (18.5; 98.0)	57.5 ± 19.5 (25.6; 99.2)
CT_f (log_{10} units)	−1.87 ± 0.14 (−2.30; −1.50)	−1.86 ± 0.18 (−2.12; −1.30)	−1.87 ± 0.15 (−2.35; −1.56)
CT_0 (log_{10} units)	1.73 ± 0.76 (0.37; 3.44)	1.63 ± 0.46 (0.66; 2.77)	1.56 ± 0.57 (0.59; 3.26)

VA: corrected distance visual acuity measured using a logMAR chart, τ: time constant for contrast recovery, CT_f: final contrast threshold. CT_0: contrast threshold elevation at time zero. Asterisk indicates significant improvement compared to vehicle-control treatment with $p < 0.05$.

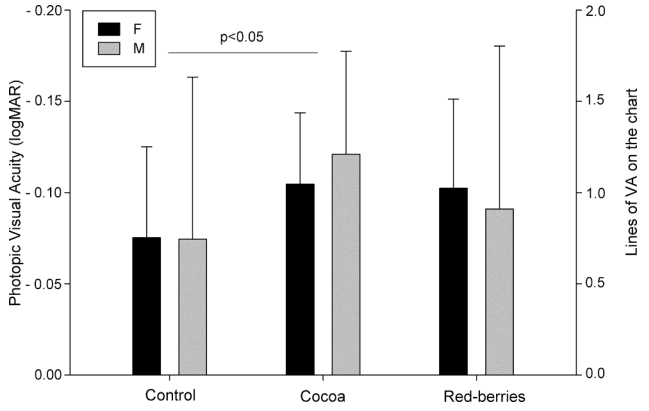

Fig. 3. Mean visual acuity measurements made under the three diet conditions (control, cocoa and red-berries) in the female and male groups. Mean VA are plotted as logarithms of the minimum angle of resolution (logMAR units) on the left y-axis and as numbers of lines on the chart on the right y-axis.

affected by any of the two polyphenol diets.

The effect of polyphenols on visual acuity has been scarcely addressed in the literature. In our study an improvement in the photopic VA values was observed with cocoa diet but showed only a non-significant trend to an improvement with red-berries diet. Our result is in agreement with a recent study that also showed that VA improved by 4 letters on the VA chart after consumption of a dark chocolate bar compared with a milk chocolate bar (Rabin et al., 2018). This improvement was attributed to increased blood flow because the effects of flavanols on visual function has been proposed to rely on flavanol-induced nitric oxide–dependent vasodilation (Fisher et al., 2003). However, the authors did not show any direct evidence of the causality. Furthermore, the natural presence of theobromine in dark chocolate and not in white chocolate was not taken into consideration. In our study, methylxanthines in 3-hours urine were measured after cocoa and red-berries ingestion. Taking into account that higher levels of theobromine were found only for the cocoa diet it could be that theobromine and not the flavanols in cocoa are the cause of the improvement of VA after cocoa ingestion. However, the fact that a trend to a similar improvement of VA with red-berries was found make it also possible that polyphenols and theobromine work in a synergistic way potentiating the observed effects in VA. It should be noted that the effects of caffeine or theobromine on the eye are not well known. Caffeine is expected to show activity in all structures within the eye since it has been shown to be diffusible (Yoon & Danesh-Meyer, 2019), improves performance on simple and complex attention tasks in humans (Einöther & Giesbrecht, 2013) and improves contrast sensitivity of freely moving rats (Tsunoda, Sato, Kurata, Mizuyama, & Shimegi, 2019). A cocoa and caffeine plus theobromine combination has shown improvements on reaction time, spatial attention and visual information processing (Field et al., 2011; Karabay et al., 2018; Smit, Gaffan, & Rogers, 2004). Therefore, the improvement in VA found in our study could be attributed to an improvement in attention and/or visual information processing due to cocoa flavanols and the methylxanthines theobromine and/or caffeine that, together with the expected effect of polyphenols on vasodilatation could explain the better performance of cocoa than red fruits in ameliorating VA values.

Another important fact that should be taken into consideration when interpreting our results is the fact that we decided to use a very low amounts of flavanols and anthocyanins in our intervention in order to use amounts of cocoa or red-berry powder that were realistic from a practical point of view and feasible in a daily balanced diet. The few studies in the literature on visual health used much higher flavanols doses and this might explain the differences in results found within our work.

Regarding the mechanisms by which the visual function can be improved by flavonoids, while research on rabbit eyes suggests that flavonoid intake increases ocular blood flow (Park & Chiou, 2004) it is not clear if this biological effect of flavonoids is translated into an improvement in visual function in humans. Recently, a small crossover trial failed to demonstrate any effect of flavanol-rich dark chocolate on automatically assessed retinal blood flow (vessel density) on optical coherence tomography (OCT) angiography or VA and large-letter contrast sensitivity in 22 healthy participants that consumed 20 g of dark chocolate containing 400 mg of flavanols or 7.5 g of milk chocolate (Siedlecki et al., 2019). Testing visual function under low luminance conditions, when the oxygen consumption in the retina increases (Birol, Wang, Budzynski, Wangsa-Wirawan, & Linsenmeier, 2007), is a good strategy to find out if the effect of anthocyanins and flavanols on vasodilatation is translated into an improvement in visual function. In our study, means for low luminance or mesopic VA and mesopic pupil size did not vary significantly between the three diet groups. This is in agreement with other study that also failed to find any effect of bilberry on low luminance VA or contrast sensitivity using a high dose of bilberry anthocyanins for a longer period (Muth, Laurent, & Jasper, 2000)

Additionally, in our study higher levels of urinary TPC were found after the ingestion of cocoa flavanols or red-berries anthocyanins in comparison with the vehicle-control. In general, baseline or control TP might be increased than expected due to the pre-study diet and the presence of anthocyanin and flavanol rich foods in it. In our study, this potential confounder due to a normal, healthy human diet of foods that naturally contain polyphenols (Morris & Tangney, 2011) was controlled through 24-h dietary questionnaires that showed no significant differences in total energy or nutrient intake between study days for all participants. Interestingly, when data on TPC were stratified by sex we found differences between diets for the entire population. Differences in TPC by sex also presented significant differences. As the interaction between both variables was not significant, it meant that both sexes had a similar behaviour regarding differences by diet. Even if PTC baseline values where statistically significant higher for females than for males, the differences of cocoa and red-berries diets operate the same way in each sex.

To our knowledge, this is the first study to examine the effects of cocoa flavanols or red-berries anthocyanins on the time course of DA measured psychophysically through contrast detection. As far as we know, studies that have examined the effect of anthocyanins on rod-mediated DA used the conventional luminance-based approach and most results only showed the final threshold instead of the rate of recovery. These studies failed to demonstrate a significant effect of anthocyanins on DA in healthy eyes (Canter & Ernst, 2004; Kalt et al.,

2014; Zadok, Levy, & Glovinsky, 1999). Additionally, in the present study, cocoa and red-berries did not have an effect on contrast recovery time for cone-mediated DA (time to dark-adapt) nor mesopic final CT. The photoreceptors have an extraordinarily high energy demand, and maintenance of photoreceptor homeostasis and function requires sufficient tissue oxygenation from the adjacent vascular structures (Wong-Riley, 2010). In our study, an acute dose of cocoa flavanols or red-berries anthocyanins was insufficient to improve DA in young healthy eyes with good homeostasis per se. A normal supply of oxygen and nutrients to the retina, essential to maintain good vision, could be altered in aging and neurodegenerative diseases of the retina such as age related macular degeneration and diabetic retinopathy where DA was found to be impaired (Dimitrov, Guymer, Zele, Anderson, & Vingrys, 2008; Jackson, Owsley, & McGwin, 1999; Jackson, Scott, Quillen, Walter, & Gardner, 2012; Owsley, Jackson, White, Feist, & Edwards, 2001). Therefore, it is still to be elucidated whether flavanols or/or anthocyanins could play a role in eye conditions with altered supply of oxygen and nutrients to the retina and further research is still needed in this sense.

In the present study, CTs were measured using sinusoidal gratings of low-spatial-frequency at mesopic light level to examine visual contrast mechanisms mediated through inner retinal cells (Lee, 2011). The mean CTf was not affect by polyphenol diets in agreement with other studies. In a multiple dose oral co-administration of a high dose of anthocyaninns failed to improve mesopic contrast sensitivity in healthy subjects (Muth et al., 2000; Zadok et al., 1999). Additionally, large-letter or low-spatial frequency contrast sensitivity remained unchanged after dark chocolate ingestion in young healthy subjects (Rabin et al., 2018).

Taking into account our results together with the existing literature we can conclude that more studies in larger groups are needed to confirm the health effects of, cocoa, and flavanols in the eye.

5. Conclusion

In healthy eyes, photopic VA improved significantly after cocoa flavanols ingestion and showed a non-significant trend to an improvement with red-berries anthocyanins. In parallel, TPC in 3-hours urine was increased for both cocoa and red-berry diets in relation to the control diet. Theobromine levels in 3-hours urine were increased only for the cocoa diet. However, mesopic VA was not affected by any of the polyphenolic diets assayed in our conditions. Contrast recovery time for cone-mediated DA and final CT were unaffected by cocoa flavanols or red-berries anthocyanin. This work highlights the need for new research that delves deeper into the effect of flavanols, anthocyanins and methylxanthines on visual acuity and attention, both in acute and chronic interventions.

6. Ethics statement

The research project was approved by the Ethics Committee of the San Carlos Clinic Hospital (C.I. 18/510-TFM_R_X) and by the Bioethics Committee of CSIC (141/2018). All subjects signed a written informed consent prior to their participation in the study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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